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In re application of:

Olle KORSGREN et al

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NOVEL USE WITH TRANSPLANTATION SURGERY

Examiner: **Donna A. Jagoe**

Art Unit: **1614**

APPEAL BRIEF

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Ex parte Copping, 180 USPQ 475, 476 (POBA 1972)

Impax Laboratories, Inc v. Aventis Pharmaceuticals Inc., Fed.Cir., No. 2007-1513, 10/3/2008

The present appeal is taken from the final rejection mailed May 22, 2008, of claims 4, 8, 9, 11, and 27. A clean copy of these claims, double spaced, appears in the appendix to this brief.

REAL PARTY IN INTEREST

The assignee of the present application Corline Systems AB of Uppsala, SWEDEN.

RELATED APPEALS AND INTERFERENCES

There are no known related appeals or interferences.

STATUS OF CLAIMS

Claims 4, 8, 9, 11 and 27 are rejected, and all of these claims are appealed.

Claims 14 and 26 are withdrawn from consideration.

Claims 1-3, 5-7, 10, 12, 13 and 15-25 are canceled.

STATUS OF AMENDMENTS

All amendments have been entered.

SUMMARY OF CLAIMED SUBJECT MATTER

The present invention, as recited in the two independent claims 4 and 27, relates to the transplantation of insulin producing cells into a patient suffering from insulin dependent diabetes mellitus (IDDM), wherein the cells (islets) so transplanted have been modified by irreversible adsorption with heparin or a fraction or derivative thereof onto the surfaces of the individually isolated islets. This coating takes place from an aqueous solution of the heparin, e.g. Corline Heparin Conjugate, with the result of obviating the significant problem of clotting.

It will be pointed out in the "argument" section below that it was the applicants who discovered that clotting was a major problem as to why islet transplantation has been unsuccessful in the past.

I. -Concise Explanation of the subject matter of Independent claim 4.

Claim 4, in its first paragraph, calls for a method comprising transplantation of insulin producing cells in the form of individually isolated islets to a patient suffering from insulin dependent diabetes mellitus (IDDM):

The first paragraph of the appellants' specification states that the invention "is with the field of transplantation surgery" and "relates to use of a clotting preventing agent in the production of a drug for administration in association for transplantation of cells…, such as insulin producing cells to patients with insulin dependent diabetes mellitus, IDDM." Also see the top paragraph at page 4 of the Appellants' specification.

That the islets or cells are "isolated" is explicit in the second paragraph on page 3, and the fourth line of example 1 on page 6; and that "isolated" means "individually isolated" is confirmed in the declaration of independent expert Professor James Shapiro, MD, PhD, executed December 8, 2007, and filed in the present application with the reply of December 10, 2007. It is also confirmed in the third declaration of co-invention Rolf Larsson, PhD, executed November 29, 2007, and also filed with the reply of December 10, 2007, noting especially paragraph (5).

In its second paragraph, claim 4 recites that the "individually isolated islets are modified by irreversible absorption with a clotting inhibiting agent comprising heparin or a fraction or derivative thereof onto the surfaces of the islets"; and the third paragraph on page 3 of Appellants' specification mentions that "the clotting preventing agent is an anticoagulant, such as heparin or fractions or derivatives thereof." In the immediately following paragraph (fourth

paragraph on page 3) it is stated that the "islet cells are coated with heparin or fractions or derivatives thereof by preincubation of islets in a solution containing heparin or fractions or derivatives thereof" and further mentions the use of "a conjugate of heparin to coat the islets." That the isolated islets are modified by irreversible absorption is supported by the first sentence of example 3 at the bottom of page 9 of Appellants' specification.

In its third paragraph, claim 4 further recites that the "individual cells are each separately coated with heparin or a fraction or derivative thereof by pre-incubation of islets in an aqueous solution containing heparin or a fraction or derivative thereof":

Again, the fourth paragraph on page 3 of Appellants' specification indicates that the islets are coated with heparin or a conjugate thereof, and also mentions that this is "by preincubation of islets in a solution...." Reference to "soluable heparin" as in the title of example 1 (page 6) means an aqueous solution as indicated in the sentence spanning pages 9 and 10 (example 3) and the fact that Corline Heparin Conjugate, as disclosed in WO 93/05793, incorporated by reference into the present specification in the second paragraph on page 6, comprises an aqueous solution.

Claim 4 concludes with reciting that the "clotting inhibiting agent acts to inhibit clotting or reduce clotting":

The fourth paragraph on page 3 mentions the effect of the reduction of clotting, and the top paragraph on page 4 mentions the prevention of clotting. Also, again please see the second paragraph on page 6, as well as the experimental results shown in the working examples.

II. -Concise Explanation of the subject matter of Independent claim 27.

The preamble for claim 27 is the same as that of claim 4, and so the concise explanation of the preamble is respectfully repeated by reference to that submitted above.

The second paragraph of claim 27 is the same as the second paragraph of claim 4, except for a typographical error, i.e. in the third line of the second paragraph of claim 27, the word "thereon" should be "thereof". As the concise explanation for the second paragraph of claim 27 is the same as that for claim 4, the concise explanation of such second paragraph of claim 4 is respectfully repeated by reference.

The third paragraph of claim 27 recites "said modification comprising incubating said islets in a solution of heparin or a fraction of derivative thereof." Another typographical error appears here in that the word following "fraction" should be "or", rather than "of". Appellants' would hope to be able to correct the typographical errors in this claim.

At any rate, incubating the islets in a solution of heparin is clearly supported in the fourth paragraph on page 3 of Appellants' specification.

Like claim 4, claim 27 concludes with the recitation that the "clotting inhibiting agent acts to inhibit clotting or reduce clotting", supported for example by the fourth paragraph on page 3 and the top paragraph on page 4 of Appellants' specification.

GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

Main claims 4 and 27, and dependent claims 8 and 11 are rejected under Section 102 as anticipated by Wagner et al, DE 196 23 440 (Wagner).

Main claims 4 and 27, and dependent claims 8 and 11, are also rejected under Section 102 as anticipated by Soon-Shiong et al, USP 5,705,270 (Soon-Shiong).

Main claim 4 and dependent claims 8 and 11 are rejected under Section 102 as anticipated by Nomura et al, "Unpurified islet cell transplantation in diabetic rats," Transplantation Proceedings, Vol. 28, No. 3 (June , 1996), pp. 1849-1850 (Nomura).

Claim 9 is rejected under Section 103 as obvious from Soon-Shiong "and" Wagner "as applied to claims 4, 8, 11, and 27…, and further in view of Couser et al", "The Effects of Soluable Recombinant Compliment Receptor I on Compliment-Mediated Experimental Glomerulonephritis", Journal of the American Society of Nephrology (1995), Vol. 5, No. 11, pp. 1888-1894 (Couser).

ARGUMENTS

Appellants' respectfully maintain that the references applied under Section 102 do not anticipate any of appellants' claims, and that the proposed combination or combinations applied under Section 103, even if such combination or combinations were obvious, would not result in the claimed subject matter.

I. -Background

As pointed out in the second paragraph on page 1 of Appellants' specification, transplantation of isolated islets has proven to be considerably less successful compared to whole pancreas transplantation, and yet "there is no obvious immunological explanation as to why transplantation of whole pancreas is more successful than islet transplantation."

As pointed out in the bottom paragraph on page 2 of Appellants' specification, the present invention is based on experiments performed by the Appellants "implying adding human, adult porcine or fetal porcine islets to human whole blood" and being "struck by the vigorous coagulation occurring when these islets were injected into human ABO-compatible blood." Based on their microscopical examinations, it became evident to them "that the islets are rapidly coated by a layer of platelets which soon develops into an organized thrombus." This biological event has previously not been considered and

is now suggested to be a major explanation as to why the outcome of autologous islet transplantation has been comparatively unsuccessful." The present invention is based on this new discovery.

II. -What the prior art discloses

The Wagner disclosure is not very clear. Nevertheless, the following text appears on page 4 of the Wagner translation near the bottom of the page:

> Normoglycemia with the objective of a timely and need-based release of insulin is possible only by a biological replacement of the insulin producing islet cell apparatus through the pancreas or by **islet cell transplantation**. Free allogenic transplants require intensive immunosuppression. This is why it could possible to develop "bio-artificial pancreas" successfully in a growing number of patients by a biological replacement of an organ i.e. by **using isolated islet cells of a pig**. In addition to this, above mentioned immunosuppression can be prevented by using immuno-separation membranes. [Emphasis believed to be that of the Examiner]

From this, it appears that Wagner wishes to develop a "bio-artificial pancreas" by encapsulating isolated islet cells of a pig using immuno-separation membranes, and this is further mentioned on page 5 of the translation under the headings "Objective" and "Bio-artificial pancreas":

> Insulin producing cells that react to glucose stimulants by a timely or need-based release of insulin are implanted in a diabetic organism. The artificial membranes protect the free islet cells of allogenic or kenogenic origin from being destroyed by the

> immune system of the receiver. Two different forms of membranes based on diffusive soft transport that are being discussed presently.
>
> 1. Macro-capsulation is capillary membranes (Diagram 1): Here, the islets are capsulated into capillary membranes with diameters ranging from **0.5 to 4 mm and length between 2 and 3 cm** and these are then implanted in the free abdominal cavity. [Emphasis added]
>
> ...
>
> 2. Micro - capsulation: In micro - capsulation, individual islets are enclosed in the smallest possible capsules made of alginate complexed with polylysine and transplanted. The microcapsules have a diameter of **0.5mm**. This technique is very popular because experiments on animals have shown that this technique increases the survival time of the transplant substantially in comparison to the non-capsulated islets in the control tests. The results however cannot be reproduced in a uniform manner...[Emphasis added]
>
> 3. Most of the microcapsules of a diameter of half a millimeter have a volume several times larger than that of the islets of Langerhans, **which are 50 - 300 µm in size**. Narrower the membranes are to an islet, or the smaller a microcapsule is, the better they are for nourishment and insulin - release at the expected conditions. This also means that thin and closely fitting membranes can be implanted in other compartments with better oxygenation. [Emphasis added]

It is clear that Wagner is disclosing encapsulation of the islets, that the microcapsules have a diameter of 0.5 mm, and "have a volume several times larger than that of the islets…, which are 50-300 µm in size."

At the bottom of page 11 of the translation, there is mention of "pharmacotherapy with anticoagulants, platelet

aggregation inhibitors, and plasminogen activators." No mention is made of heparin until claim 7 which says that, in accordance with claim 6 which calls for the "product" being "characterized by the fact that the immobilization system contains components, which either suppress or prevent agglomeration of the blood", that heparin (or other materials) are used to antagonize agglomeration." There is no disclosure as to how this is done.

Page 23 of the Wagner translation appears to discuss the types of polymers used for encapsulation and mentions polyethylene oxide. Polymer films of various types are mentioned at the bottom of page 24 and the top of page 25 of the Wagner translation.

The only experiments described in Wagner appear to be based on the first form of membranes, namely capillary membranes (diagram I), and these experiments are described beginning at the bottom of page 26 of the translation in which it is mentioned that the functions of the islet cells are observed in "silicon" (silicone?) catheters.

To summarize, Wagner describes microcapsules used in transplantation surgery. The microcapsules are made of organic material (polylysin complexed alginate) and allow release of active substances, in particular insulin and insulin related substances. These capsules are filled with islets of Langerhans, and the objective is to have a controlled release of insulin through the microcapsules. The

disclosed microcapsules are approximately 0.5 mm in diameter, and have an inner volume which is many times larger than the volume of the encapsulated islets (see page 6, line 10 and page 7, lines 6-8, of the English translation).

It should be noted that Wagner discloses no other alternative than encapsulation (see for example page 5, line 7-10 and page 6, line 7-9, of the English translation). In particular, it should be noted that despite the fact that there is a discussion of the degree of free space inside the capsules and the effect thereof on e.g. the diffusion behavior, the only alternative suggested for enhancing the efficiency is to make the capsule smaller (see page 7, lines 9-13, of the English translation). Nowhere in Wagner is it even remotely suggested that the islets be coated in the sense of the present invention.

Soon-Shiong describes the encapsulation of islets by coating with polymerizable alginate or with a composite of alginate and polyethylene glycol. Heparin is mentioned in the Soon-Shiong specification only at column 6, line 60, among a basket or shot gun disclosure of biocompatible materials including polymerized saccharides such as alginates.

Soon-Shiong contains thirty-two examples, none of which mention heparin.

In the bottom paragraph of column 7 of Soon-Shiong, it is mentioned that a particularly preferred embodiment

utilizes a modified alginate capable of being polymerized and ionically cross-linked as the encapsulating polymer, wherein the alginate is modified to produce the compound A-X where A is a naturally occurring or synthetic modified form of alginate, and X is a moiety containing a pair of carbon atoms separated by a double or triple bond and capable of undergoing free radical polymizeration. Claim 5 specifies that when A is covalently linked to Y, A may be a polysaccharides selected from a group of materials including heparin.

Nomura describes a study undertaken "to evaluate the effects of various anticoagulants" including heparin "on portal vein pressure, recipient survival, and graft survival when unpurified islets are transplanted into the portal vein using the isograph model."

Under the heading "Materials and Methods" the following text appears:

> Unpurified islets were transplanted into the portal vein through a 24-gauge cannula over three minutes. Fifteen minutes after the start injection of the cells, portal vein pressure was measured. Recipients were divided into four groups.

Heparin or heparin plus other materials were "injected into the portal vein…."

The third declaration of Rolf Larsson, Ph.D., one of the co-inventors of the present invention, which Declaration was executed November 29, 2007, and filed with the reply of

December 10, 2007, states in paragraph (14) on page 5 as follows:

> (14) Newly relied upon Nomura…discloses only the use of heparin administered systemically. Systemic administration of heparin is likely to generate bleeding complications, and has nothing to do with our invention which relates to the use of surface-bound heparin which acts locally on the surface of the islets thus eliminating bleeding complications.

It is seen that Nomura does not disclose any treatment of the islets by incubation with heparin.

Couser relates to the systemic administration of a drug, and not to any transplantation of insulin producing cells. This publication appears to have nothing to do with either the present invention or any of the other cited references.

III. -Claims 4, 8, 11 and 27 define novel subject matter over Wagner.

Interpreting the difficult to understand Wagner as closely as possible to the present invention, a critical distinction is that the closest Wagner comes to the present invention is encapsulating islets within a polymer shell or capsule having a diameter of 0.5 mm, "a volume several times larger than that of the islets…, which are 50-300 μm in size." In the present invention, the islets are not encapsulated, but

are instead treated with a clotting preventing agent such as heparin.

Thus, contrary to claims 4, 8, 11 and 27, Wagner does not disclose "transplantation of insulin cells in the form of individually isolated islets…."

Contrary to claims 4, 8, 11 and 27, Wagner does not disclose that the "individually isolated islets are modified by irreversible absorption with a clotting inhibiting agent comprising heparin or a fraction or derivative thereof onto the surfaces of the islets,…."

Contrary to claims 4, 8 and 11, Wagner does not disclose "individual islets cells… each separately coated with heparin or a fraction or a derivative thereof by pre-incubation of islets in an aqueous solution containing heparin or a fraction or derivative thereof,…; or as recited in claim 27, the "modification comprising incubating said inlets in a solution of heparin or a fraction [or] derivative thereof."

And, unlike the present invention "wherein said clotting inhibiting agent acts to inhibit or reduce clotting", there is no evidence whatsoever that anything disclosed by Wagner will produce such a result, i.e. there is no inherency because there is no reasonable certainty that any such result would be achieved in anything taught by Wagner.

As understood, the Examiner's position is that there is no difference between the encapsulation option of Wagner, in which heparin may optionally somehow be involved, and the

subject matter of Appellants' claims 4, 8, 11 and 27, but this is speculation on the part of the Examiner which not only goes far beyond any disclosure of Wagner, but which also is contrary to the evidence, including the declarations of record in this case.

Appellants submit and respectfully represent that it is clear to anyone skilled in the art upon reading Wagner that the capsule material is utilized as a measure to avoid immunological reactions, due to the fact that the capsule material exhibits poor compatibility with blood. The closest Wagner disclosure to the present invention is in the blending of the capsule material with heparin in claims 6 and 7. But there is no disclosure and/or teaching by Wagner to indicate that the islets per se might provoke clotting, which is an important discovery upon which the present invention is based. Wagner does not provide a disclosure which would enable one skilled in the art to practice appellants' claimed invention, *Impax Laboratories, Inc. v. Aventis Pharmaceuticals Inc.*, Fed.Cir., No. 2007-1512, 10/3/08.

The closest that Wagner comes to the present invention is in Wagner's claims 6 and 7 wherein, as best can be understood, Wagner teaches mixing of the anticoagulant, e.g. heparin, in the material used to form the capsules. The only way that one can read Wagner on Appellants' claims is by superimposing Appellants' disclosure on Wagner. Wagner simply does not disclose modifying the cells by irreversible

absorption of the clotting inhibiting agent as Appellants have claimed.

Contrary to the Examiner's position, there is a fundamental difference between encapsulation (the closest Wagner comes to the present invention) and modification by irreversible absorption of a clotting inhibiting agent in accordance with the present invention and as claimed. As supporting evidence, Appellants respectfully invite attention to the first declaration filed in this application on March 2, 2004, in the name of three of the inventors, namely Olle Korsgren, MD, PhD; Bo Nilsson, MD, PhD; and Rolf Larsson, PhD. This declaration states in part as follows:

> First, and in a general way, we can state as fact that our invention as set forth in the present application is not based on the same principles, i.e. encapsulation, as the Wagner et al citation DE 196 23 440 A 1 (hereinafter "Wagner") or the Soon-Shiong et al citation U.S. patent 5,705,270 (hereinafter "Soon-Shiong").

The declarence then provides more detail:

> The U.S. Examiner has stated (Advisory Action), "Coating and encapsulating appear to be the same." And that, "this appears to be a difference in nomenclature only." We state as fact that coating according to our invention is absolutely not the same as encapsulating according to Wagner and Soon-Shiong. Coating in accordance with our invention of the present U.S. Patent application does not result in encapsulation, but instead results in a linkage between the islets and the heparin or other clotting preventing agent, i.e. the "coating" according to our invention results in the isolated islets being modified by irreversible adsorption with the heparin, a

> physical condition which is entirely unlike encapsulation with a polymeric material as disclosed by Wagner and Soon-Shiong.

Microcapsules are, as disclosed and taught by Wagner, much larger than the islets, Wagner having stated at page 7 of the translation: "Most of the microcapsules of a diameter of half a millimeter have a volume several times larger than that of the islets…, which are 50-300 μm in size." The above noted first declaration states on page 3 as follows:

> Such microcapsules, including those produced according to the methods of Wagner and Soon-Shiong, consist of polymer spheres of 400-800 μm in diameter, typically made of alginate (a polysaccharide) or synthetic polymers, with a wall thickness of 10-50 μm to separate the encapsulated islets from their biological environment, the ultimate goal of the capsule shell being to establish an immunological barrier. Encapsulation implies a coherent material in the form of a sphere which is not integrated into the biological surface of the islets but rather holds a number of islets being dispersed in the interior of the spheres.

And, in the paragraph spanning pages 3 and 4 of the first declaration:

> The references of Wagner and Soon-Shiong therefore are very clearly concerned with encapsulation techniques, very different from our technique. As an example, both references teach the use of alginate as a vehicle to construct microcapsules. The encapsulation techniques according to Wagner and Soon-Shiong result, according to what is desired and clearly implied in these documents, in the islets being trapped and physically enclosed within the microspheres, i.e. within the capsule shells. As indicated above, this is a

> physical state which is quite different from what occurs according to our method.

Thus, no encapsulation occurs in accordance with the present invention.

As best understood, the Examiner believes and has taken the position that Corline Heparin Conjugate used in example 3 of the present application, having a polymer component, is therefore no different from Wagner, but this is not correct, because use of Corline Heparin Conjugate as in appellants' example 3 does not lead to encapsulation. Please see page 5 of the aforementioned first declaration, where the following statement of fact appears:

> In the case where the islets have been modified by e.g. the Corline Heparin surface (e.g. our Example 3), there are no prerequisites that would lead anyone skilled in the art to conclude that such a procedure involving simple mixing would represent encapsulation. The procedure implies (and results in) attachment to the biological structure of the islets of individual high-molecular weight molecules, with no semi-permeable function.
>
> ... In our invention, contrary to Wagner and Soon-Shiong, no such capsule is formed. The heparin in our invention does not encapsulate the islets. Even when a heparin-conjugate with alginate, e.g. the Corline heparin conjugate of Example 3 of our above-identified U.S. Patent application, is used, encapsulation of the islets does not occur.

And, the Declarants add at pages 6 and 7, the following:

> We state as fact that it is inherent in our above-identified U.S. patent application that the surface of each individual islet is modified to reduce thrombogenicity, and each

> islet is free to interact with the biological environment. No physical barrier and no immunological barrier heparin coating occurs in our invention. On a molecular level there is provided a thickness of heparin of at most 0.1 μm, which is non-coherently attached directly to the biological surface of the islets.
>
> Artificial encapsulation implies that a synthetic polymer is used to establish a physical barrier between the islets and the biological environment (tissue, blood, etc.) only to allow certain substances (e.g. insulin) to pass across the barrier. Our process of the present application may be referred to as non-artificial based on the fact that the islets are free to fully interact at a molecular scale with their biological environment and that they retain their capacity to release insulin without any passage through a semi-permeable membrane. The purpose of attaching e.g. heparin to the surface of the islets is entirely to down-regulate the tendency of the islets to induce coagulation and inflammation.
>
> We therefore state as fact that our islets, after "coating" with heparin, are not encapsulated.
>
> Our heparin-modified islets can be obtained by simple mixing of heparin or heparin complex with the islets, as in Example 3 of our U.S. patent application. On the other hand, in Wagner and Soon-Shiong there are required operations, which are the main focus of these documents, for the creation of the capsule shells, e.g. extrusion in a two-phase coaxial flow system according to Example 20 of Soon-Shiong, or an emulsification technique with a photo polymerization as set forth in Example 19 of Soon-Shiong.

What appears in a declaration is of course evidence, and the Examiner has no evidence to the contrary. Throughout prosecution of this application, the Examiner has essentially

disregarded the declarations in spite of the fact that the Declarants are experts in the art who are entitled to present expert opinion which must be considered, and further in spite of the fact that the Declarants submitted statements of fact based on their knowledge.

Appellants' position is supported by *In re Khelghatian*, 150 UPSQ 661,663, footnote 2 (CCPA 1966), wherein Judge Rich, speaking for the Court, and commenting on the Supreme Court's decisions in *Graham v. John Deere Co.*, 383 U.S. 1, 148 USPQ 459, and *United States v. Adams*, 383 U.S. 39, 148 USPQ 479, stated as follows: "In our view the Court there said nothing at all about 'doubtful cases,' nor in any way suggested that any record evidence should not be accorded its full probative weight…. Such an approach [brushing off a Declaration because the Examiner "is satisfied" that the invention is unpatentable] is reminiscent of the proverbial "don't bother me with the facts, my mind is made up" method of decision and has, we think, no place in the application of 35 U.S.C. 103. We therefore remain of the view that the law requires consideration of all ***evidence***, properly submitted, …." (emphasis in original; bracketed material added). Thus, all evidence is to be considered.

Also see *In re Alton*, 37 USPQ2d 1578, 1583 (Fed Cir 1996), relating to declarations of fact. Also see *Ex parte Copping*, 180 USPQ 475, 476, relating to opinion affidavits from experts.

Wagner discloses and seemingly denigrates a prior micro-capsulation system where the islets are enclosed in the smallest possible capsules (diameter of 0.5 mm) made of alginate complexed with polylysine, noting the English translation of the Wagner description on page 6, lines 7-10. The islets are much smaller (50-300μm), see pg. 7, line 8), so many fit within the micro-capsules. Wagner teaches providing an "artificial surface" forming a "mono-layer film" over "proteins" which have "selectively accumulate[d], noting page 13 at the bottom.

Wagner teaches the use of encapsulation, e.g. in the trial commencing at the bottom of page 27, 3000 islet cells were encapsulated on two microprotein silicon catheters. This implies that a definite number of cells (typically several hundred) are confined in a capsule, namely a compartment, defined by a semi-permeable membrane. An objective and apparent result of Wagner is platelet aggregation of blood (pg 17-19 of Wagner). This is distinctly different from the presently claimed invention which involves and teaches surface modification of each individual islet without changing the physical configuration of the islet.

The preferred route for transplantation of islets of Langerhans is to inject the isolated islets into the portal vein allowing the islets to settle in the liver. The result from an animal experiment where porcine islets were intraportally transplanted into pigs is found in appellants'

specification at page 5, lines 13-20. The method of intraportal transplantation can also be used in humans, and thus it is impossible to use the intraportal route if the cells have been enclosed in capsules of high molecular weight material that are considerably larger in size than the individual cells/islets.

Contrary to Wagner, in the present invention the heparin or modified heparin will be attached to each individual islet without changing the dimensions, i.e. the size which again is completely different from Wagner wherein the capsule is much, much larger than the size of individual cells.

As further evidence, the appellants submitted a second declaration, executed February 4, 2007, and again in the names of Dr's Korsgren, Nilsson and Larson, filed with the Reply of April 4, 2007, in which they stated as fact:

> Encapsulation (also referred to as microencapsulation) implies that the islets are confined within a polymer membrane that is not in direct contact with the islets. Extensive efforts have been spent on preparing microcapsules primarily using alginates as the capsules-forming material in accordance with what is disclosed in Wagner and Soon-Shiong.
>
> A recent article (ref. 1), Dufrane et al[1], is representative for the current state-of-the-art. As illustrated in Fig 1 in the

[1] 1. Dufrane D., Goebbels R-M., Saliez A., Guiot Y., and Gianello P.: Six-months survival of microencapsulated pig islets and alginate biocompatibility in primates. Proof-of-concept. Transplantation, 9, 1345-1353, 2006

> cited article, an optimal capsule should be a sphere with a diameter of 650- 700 μm. Wagner specifies a diameter of 500 μm (p. 6, item 2, l.3-4 in translated document).
>
> Considering that the islets have a size of 50-300 μm, we state fact beyond any doubt that one or several islets will be enclosed in each microcapsule with considerable dead space between the islet(s) and the enclosing membrane.

The Declarants then stated that even with the most advanced current technology "capsules of the prior art leave a dead space of 25-50 μm between the cell surface and the polymer membrane…." As a result, the "dead space creates delayed response times due to the fact that glucose must first defuse through the membrane, and then the glucose has to be transported by a concentration gradient across the dead space until it reaches the cell surface."

The Declarants continue near the bottom of page 3 of such declaration, as follows:

> On the contrary, in our invention, the material used, e.g. Corline Heparin Conjugate, is adsorbed directly in close contact with individual islets with no dead space, whereby glucose in blood interacts directly with the islet cells so that a physiological response with regard to release of insulin is achieved. The following picture (Fig. 2), prepared in our laboratory, shows one islet cell coated with heparin according to our invention and examined by confocal microscopy using fluorescently labelled antithrombin that binds to heparin. It is evident that the coating follows the contour of the cell and is in direct contact with the cell surface.

This declaration then presents a photomicrograph, Fig. 2, which shows that the islets treated according to the present invention have the heparin **on the surface of the islets.**

Appellants believe and respectfully submit that this is strong evidence which the Examiner has not and cannot rebut.

This second declaration then moves to another distinction at page 5, where the following statement appears in which the insoluble nature of the microcapsules of Wagner which form a membrane barrier, is contrasted with the present invention:

> There is another distinct difference between encapsulated islets such as those of Wagner and Soon-Shiong, and heparin coated islets according to our invention that needs to be emphasised. The spherical membrane that encapsulates the islets of the prior art is an insoluble polymer with a cross-longitudinal network of bonds (thus it forms a membrane barrier between the islet surface and the surroundings), whereas the material we use, e.g. Corline Heparin Conjugate, is a soluble macromolecule that is adsorbed as discrete molecules onto the cell surface with no cross-longitudinal linkages being formed between the conjugate molecules (thus no membrane barrier is formed between the islet surface and the surroundings).

The second declaration concludes as follows:

> As clearly explained in our previous Declaration, the encapsulating membrane composed of an insoluble polymer proposed by Wagner et al and Soon-Shiong et al constitutes a barrier to immunologically active cells and molecules, whereas no such barrier is created by adsorption of the heparin, e.g. Corline Heparin Conjugate. We reiterate with strong emphasis that it is

> absolutely clear and certain that the chemical constitution and diffusion characteristics are fundamentally different between the two methods, and that there is no chemical or technical rationale on basis of which one could accurately maintain that adsorption of the Corline Heparin Conjugate would lead to encapsulation in the sense of Wagner et al and Soon-Shiong et al.

Appellants again respectfully note there is no evidence to the contrary.

Further evidence in support of appellants' position exists in the form of the third declaration of Professor Larsson executed November 29, 2007, and the declaration of independent expert James Shapiro MD, PhD, executed December 8, 2007, both of such declarations having been filed with the Reply of December 10, 2007.

In his declaration, Professor Shapiro states as follows:

> I understand from reading the specification of the above-identified application 09/890,936 that the individually isolated islets are treated with a clotting inhibiting agent, e.g. heparin or soluble Corline heparin conjugate, which is adsorbed onto the surface of the individual isolated islets. This adsorption of clotting inhibiting agent onto the individual isolated islets is quite different from islet encapsulation, the latter of which refers specifically to immunological isolation of islets from attacking immune damaging cells.

The adsorption of clotting inhibiting agent "is quite different from islet encapsulation."

At most, what Wagner discloses is an encapsulation of islets with an insoluble polymer shell, e.g., polyamide, polyester, polyolefin, etc., namely an insoluble barrier, something quite contrary to the present invention where the heparin material is adsorbed onto the cell surface with no formation of a barrier shell. Indeed, one end of the molecule is adsorbed onto the cell surface, and the other end protrudes out from the cell. Such molecules, e.g. heparin and Corline Heparin Conjugate, are water soluble molecules. The so treated islets do not delay insulin response by the cell, because there is no membrane barrier that has to be penetrated by glucose and insulin, as inevitably must occur in the prior art, including Wagner. Further in this regard, and in support of Appellants' position, attention is invited to page 3 of the Declaration of Dr. James Shapiro, executed December 8, 2007, and filed with the reply of December 10, 2007.

The present invention differs fundamentally from Wagner in not providing an impenetrable shell around the islets.

The Examiner relies on claim 8 of Wagner, but claim 8 does not disclose the use of heparin or anything similar to heparin. Please see paragraph 8 of the third Declaration of Professor Larsson. Claim 7 of Wagner does mention heparin "used to antagonize agglomeration", but Wagner does not describe how heparin might be used in the Wagner system;

please see paragraph (9) of Professor Larsson's third Declaration.

As Dr. Larsson pointed out during the interview of June 28, 2007, the Examiner's interpretation of Wagner makes no sense, because, if cells of Wagner were first mixed with an anticoagulant and then encapsulated as proposed by the Examiner at page 4 of the Official Action of August 9, 2007, the anticoagulant could not function because the anticoagulant would then be sealed within the microcapsule. This is confirmed as fact in paragraph 11 of the Third Declaration of Professor Larsson.

As to the fundamental difference between encapsulation (the prior art) and coating, please also see the first Declaration of Drs. Korsgren, Nilsson and Larsson executed in February of 2004 and filed with a "Second Preliminary Amendment for Continued Examination" of March 2, 2004; and the second Declaration of Drs. Korsgren, Nilsson and Larsson, filed with the Reply of April 4, 2007.

There is no basis for the rejection based on Wagner, and such rejection should be reversed. Such is respectfully requested.

IV. Claim 4, 8, 11 and 27 define novel subject matter over Soon-Shiong

Soon-Shiong discloses little more than Wagner, except Soon-Shiong is much more clear. But, like Wagner, Soon-Shiong relates to **encapsulation** of the islets, something fundamentally different from the present invention as already explained above, and as established by the evidence of the declarations of record also referred to above.

Thus, Soon-Shiong discloses the microencapsulation of biological materials using, for example, a polymerizable alginate or a composite thereof with polyethylene glycol. The present invention does not involve, and appellants do not claim, any such subject matter, because the present invention does not involve or comprise encapsulation of islets of Langerhans.

It is well known that the main reason for using encapsulation is to avoid immunological reactions aiming at eliminating the need for immunosuppressive therapy. In Soon-Shiong, an encapsulation system is disclosed (see col. 3, lines 53-61, and abstract) which comprises compounds which are capable of undergoing free radical polymerization e.g. by using certain sources of light. The objective of Soon-Shiong is to provide an encapsulation which allows delivery of substances from the insides of the capsule, i.e. drug delivery or insulin secretion from encapsulated islets of Langerhans, while providing immunoprotection. The Soon-Shiong patent does

not disclose features of clotting prevention, and thus teaches away from the presently claimed invention.

Appellants respectfully add, as already noted above, that the Examiner was unjustified in ignoring the evidence of the Declarations of record where the Declarants stated as fact as follows at page 7:

> We therefore state as fact that our islets, after "coating" with heparin, are not encapsulated.
>
> Our heparin-modified islets can be obtained by simple mixing of heparin or heparin complex with the islets, as in Example 3 of our U.S. patent application. On the other hand, in Wagner and Soon-Shiong there are required operations, which are the main focus of these documents, for the creation of the capsule shells, e.g. extrusion in a two-phase coaxial flow system according to Example 20 of Soon-Shiong, or an emulsification technique with a photo polymerization as set forth in Example 19 of Soon-Shiong.

The Examiner was not justified in speculating contrary to the statements of fact in the Declarations of record.

To the extent that the Examiner seems to be relying on inherency, appellants respectfully note that reliance by the PTO on inherency in a reference requires that such inherency must be reasonably certain. For example, please see *In re Brink*, 164 USPQ 247, 249:

> Absent a showing [by the PTO] of some **reasonable certainty** of inherency, the rejection... under 35 U.S.C. 102 must fail. (emphasis added)

Also see *Ex parte Cyba*, 155 USPQ 756, 757 (1967), and *In re Oelrich*, 212 USPQ 323, 326 (1981). There is no reasonable certainty that anything done by Soon-Shiong and disclosed in Soon-Shiong would provide anything identical or even similar to the present invention. Therefore, inherency in Soon-Shiong (or Wagner) is neither inevitable nor reasonably certain, and no inherency exists which can be relied upon.

Further in this regard, although claim 5 of Soon-Shiong mentions heparin as a possible alternative compound for apparently forming a co-monomer for formation of the polymer shell used in forming the microcapsules, there is no disclosure and no teaching how heparin could be applied to individual islets. Reading Soon-Shiong, like reading Wagner, does not enable one skilled in the art to reach appellants' subject matter.

At one point, the Examiner argued that "it is unclear how the heparin-alginate conjugate of the Corline system differs from the heparin/alginate system of Soon-Shiong." It is perfectly clear that there are distinct differences. Corline's heparin-amine conjugate (not alginate) can be added directly to the culture medium without affecting the dimensions of the islets. In the description of the present invention, page 10, lines 14-20, it is concluded that the surface modification of the islets by heparin is expected to decrease or even eliminate the need for insulin injections. This clearly implies that the surface modification does not

change the insulin excretion of the islets. But according to Example 25 in Soon-Shiong, a rather elaborate procedure has to be applied involving co-extrusion and photo-crosslinking. There is no provision that the islets will remain as individual islets maintaining their original dimensions.

Soon-Shiong, like Wagner, does not disclose a method as called for in claims 4 and 27 "wherein said individual isolated islets are modified by an irreversible adsorption with a clotting inhibiting agent comprising heparin or a fraction or derivative thereof". There is nothing in either Wagner or Soon-Shiong which has anything to do with irreversible adsorption. This feature is neither disclosed in the references nor is it inherent in the references, as is further made clear in the second declaration of the inventors. Again, please also see page 3 of Dr. Shapiro's Declaration, and also paragraphs (12) and (13) on pages 4 and 5 of Professor Larsson's third Declaration, as well as the first and second Declarations of the inventors.

Appellants again respectfully note that it is fundamental that the Declarations are evidence, not arguments. What is stated as fact must be accepted, and what is set forth as expert opinion must also be accepted, in the absence of evidence to the contrary, of which there is none.

The present invention relates to and describes how to modify the surface of individual islets so they can be brought into direct contact with blood without encapsulating

the islets, without having to introduce artificial materials such as used by both Wagner and Soon-Shiong, and without causing the blood to clot. In contrast, both Wagner and Soon-Shiong mention heparin as an example of a substance to be used to reduce the deleterious effect of the artificial material used to encapsulate the islets. Both Wagner and Soon-Shiong aim to shield the islets from direct contact with the blood. There is a clear and distinct difference between the present invention and both Wagner and Soon-Shiong.

Soon-Shiong does not anticipate Appellants' claim, and the rejection should be reversed. Such is respectfully requested.

V. -Claims 4, 8 and 11 are not anticipated by Nomura

The third declaration of Rolf Larsson, Ph.D., one of the co-inventors of the present invention, which Declaration was executed November 29, 2007, and filed with the Reply of December 10, 2007, states in paragraph (14) on page 5 as follows:

> (14) Newly relied upon Nomura…discloses only the use of heparin administered systemically. Systemic administration of heparin is likely to generate bleeding complications, and has nothing to do with our invention which relates to the use of surface-bound heparin which acts locally on the surface of the islets thus eliminating bleeding complications.

Applicants' claims do not recite administering heparin. Instead, the heparin used in the present invention has been applied to the individual islets, and it is these surface-treated islets which are administered to the patient.

The rejection should be reversed, and such is respectfully requested.

VI. -Claim 9 defines novel and non-obvious subject over Soon-Shiong and Wagner in view of Couser.

The deficiencies of Wagner and Soon-Shiong have been pointed out above and Couser has not been cited to make up for those deficiencies (and indeed does not do so). Actually, Couser has nothing to do with either the present invention or the primary references. Thus, Couser is fundamentally irrelevant to the present invention as stated **as fact** in paragraph 15 of the third declaration of Professor Larsson. Couser clearly relates to the systemic administration of a drug, and not to any transplantation of insulin producing cells which have already been modified as recited in claim 4, together with the added subject matter of the dependent portion of claim 9.

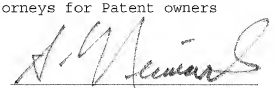
Claim 9 defines non-obvious subject matter and the rejection should be reversed. Such is respectfully requested.

CONCLUSION

Appellants respectfully submit that the Examiner has not met the burden of establishing lack of novelty for claims 4, 8, 11 and 27, or the additional burden of the establishing a *prima facie* case of obviousness for claim 9. Appellants respectfully request reversal of the rejections.

Respectfully submitted,

BROWDY AND NEIMARK, P.L.L.C.
Attorneys for Patent owners

By: 
Sheridan Neimark
Registration No. 20,520

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CLAIMS APPENDIX

4. A method comprising

transplantation of insulin producing cells in the form of individually isolated islets to a patient suffering from insulin dependent diabetes mellitus (IDDM),

wherein said individually isolated islets are modified by irreversible adsorption with a clotting inhibiting agent comprising heparin or a fraction or derivative thereof onto the surfaces of the islets,

wherein said individual islet cells are each separately coated with heparin or a fraction or derivative thereof by preincubation of islets in an aqueous solution containing heparin or a fraction or derivative thereof,

wherein said clotting inhibiting agent acts to inhibit clotting or reduce clotting.

8. The method according to claim 4, wherein more than one clotting inhibiting agent is used.

9. The method according to claim 4, wherein the clotting inhibiting agent is supplemented by an inhibitor of complement.

11. A method for increasing survival of islet cells according to claim 14, in connection with transplantation of insulin producing cells to patients with insulin dependent diabetes mellitus (IDDM), comprising inhibiting of clotting, monitored as reduced generation of thrombin-antithrombin complex (TAT complex).

27. A method comprising transplantation of insulin producing cells in the form of isolated islets to a patient suffering from insulin dependent diabetes mellitus (IDDM),

wherein said isolated islets are modified by irreversible adsorption of a clotting inhibiting agent comprising heparin or a fraction or derivative thereon onto the surface of the islets;

said modification comprising incubating said islets in a solution of heparin or a fraction of derivative thereof;

wherein said clotting inhibiting agent acts to inhibit or reduce clotting.

EVIDENCE APPENDIX

Copies of the Declarations of record, referred to above, are attached hereto. All of such declarations were entered as a matter of right.

DECLARATION UNDER 37 CFR 1.132
filed and entered March 2, 2004

SECOND DECLARATION UNDER 37 CFR 1.132
filed April 4, 2007; and entered June 5, 2007

THIRD DECLARATION OF ROLF LARSSON
filed and entered December 10, 2007

DECLARATION OF JAMES SHAPIRO, M.D., PH.D., FRCSC
filed and entered December 10,2007

RELATED PROCEEDINGS APPENDIX

There are no related proceedings in connection with the subject application.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

ATTY.'S DOCKET: KORSGREN=1

In re Application of:	)	Art Unit: 1614
	)	
Olle KORSGREN et al	)	Examiner: Donna JAGOE
	)	
Appln. No.: 09/890,936	)	Washington, D.C.
	)	
Date Filed: November 7, 2001	)	Confirmation No. 9165
	)	
For: NOVEL USE WITHIN TRANSPLANTATION SURGERY	)	

DECLARATION UNDER 37 CFR 1.132

Olle Korsgren, Bo Nilsson and Rolf Larsson hereby each solemnly declare as follows:

We are co-applicants and co-inventors of the above-identified application. We each have Ph.D. degrees and Korsgren and Nilsson also have M.D. degrees. Our CVs are attached hereto and made a part of this declaration.

Each of us is an expert in the art of the present application, and each of us is familiar with the prior art documents cited and relied upon by the U.S. patent examiner and the commentary of the U.S. examiner in conjunction with the examiner's reliance on these prior art citations.

First, and in a general way, we can state as fact that our invention as set forth in the present application is not based on the same principles, i.e. encapsulation, as the Wagner et al citation DE 196 23 440 A 1 (hereinafter "Wagner")

or the Soon-Shiong et al citation U.S. patent 5,705,270 (hereinafter "Soon-Shiong"). We elaborate below.

The U.S. examiner has stated (Advisory Action), "Coating and encapsulating appear to be the same." And that, "this appears to be a difference in nomenclature only." We state as fact that coating according to our invention is absolutely not the same as encapsulating according to Wagner and Soon-Shiong. Coating in accordance with our invention of the present U.S. Patent application does not result in encapsulation, but instead results in a linkage between the islets and the heparin or other clotting preventing agent, i.e. the "coating" according to our invention results in the isolated islets being modified by irreversible adsorption with the heparin, a physical condition which is entirely unlike encapsulation with a polymeric material as disclosed by Wagner and Soon-Shiong.

Fabrication of microcapsules surrounding individual or small clusters of islets (encapsulation) as represented by Wagner and Soon-Shiong represents a very delicate process. The procedure consists of enveloping the islets cells within homogeneous and semi-permeable artificial membranes without affecting tissue morphologic integrity or functional competence, aiming at protecting the graft from rejection in the absence of immunosuppressive therapy. Thus, the membrane

should be permeable to insulin and low molecular weight components such as oxygen, glucose, electrolytes and other nutrients and impermeable to lymphocytes and other cellular components of the immune system and also to antibodies, cytokines and other mediators of the immune system.

Such microcapsules, including those produced according to the methods of Wagner and Soon-Shiong, consist of polymer spheres of 400-800 µm in diameter, typically made of alginate (a polysaccharide) or synthetic polymers, with a wall thickness of 10-50 µm, or even more, to separate the encapsulated islets from their biological environment, the ultimate goal of the capsule shell being to establish an immunological barrier. Encapsulation implies a coherent material in the form of a sphere which is not integrated into the biological surface of the islets but rather holds a number of islets being dispersed in the interior of the spheres.

From a technical standpoint, a rather elaborate, semi-automatic procedure is required. It would be very clear for those skilled in the art that the procedures according to the present patent application are much less complicated from a technical point of view.

The references of Wagner and Soon-Shiong therefore are very clearly concerned with encapsulation techniques, very different from our technique. As an example, both references

teach the use of alginate as a vehicle to construct microcapsules. The encapsulation techniques according to Wagner and Soon-Shiong result, according to what is desired and clearly implied in these documents, in the islets being trapped and physically enclosed within the microspheres, i.e. within the capsule shells. As indicated above, this is a physical state which is quite different from what occurs according to our method.

It is well known that the main reason for using encapsulation is to avoid immunological reactions aiming at eliminating the need for immunosuppressive therapy. However, despite great efforts over two decades, there is still no encapsulation method in clinical practice, which reflects the practical and biological difficulties related to this prior approach.

The approach according to the present patent application is to rely on well-established protocols for immunosuppression, but to improve the viability of islets being injected in the portal vein of diabetic patients. There is no aim whatsoever to create an immunological barrier, as with encapsulation, but to down-regulate the inherent pro-coagulant and pro-inflammatory capacity of isolated islets. Recent research has taught us that isolated islets display a pronounced pro-coagulant and pro-inflammatory capacity which

provokes an instant blood mediated inflammatory response (IBMIR), which is distinctly different from a specific immunogenic challenge, i.e. the type of immune response triggered by transplanted cells or organs. The IBMIR leads to blood clot formation (thrombosis) which implies that a great number of islets become trapped in such thrombi and thus become non-functional.

In the case where the islets have been modified by e.g. the Corline Heparin surface (e.g. our Example 3), there are no prerequisites that would lead anyone skilled in the art to conclude that such a procedure involving simple mixing would represent encapsulation. The procedure implies (and results in) attachment to the biological structure of the islets of individual high-molecular weight molecules, with no semi-permeable function.

The U.S. Examiner has questioned what is meant by "artificial" encapsulation, and how our invention differs. We have in part addressed this above, and now add that what is "artificial" in Wagner and Soon-Shiong is the creation of an artificial shell of polymeric material, i.e. the capsule which encapsulates whatever component is intended to be encapsulated. In our invention, contrary to Wagner and Soon-Shiong, no such capsule is formed. The heparin in our invention does not encapsulate the islets. Even when a

heparin-conjugate composed of multiple heparin molecules being covalently linked to a carrier chain, e.g. the Corline heparin conjugate of Example 3 of our above-identified U.S. Patent application, is used, encapsulation of the islets does not occur.

We summarize differences between encapsulation, e.g. according to Wagner and Soon-Shiong, and heparin coating of islets according to the present invention as follows:

We state as fact that it is inherent in our above-identified U.S. patent application that the surface of each individual islet is modified to reduce thrombogenicity, and each islet is free to interact with the biological environment. No physical barrier and no immunological barrier occur in our invention. On a molecular level there is provided a thickness of heparin of at most 0.1 μm, which is non-coherently attached directly to the biological surface of the islets.

Artificial encapsulation implies that a synthetic polymer is used to establish a physical barrier between the islets and the biological environment (tissue, blood, etc.) only to allow certain substances (e.g. insulin) to pass across the barrier. Our process of the present application may be referred to as non-artificial based on the fact that the islets are free to fully interact at a molecular scale with

their biological environment and that they retain their capacity to release insulin without any passage through a semi-permeable membrane. The purpose of attaching e.g. heparin to the surface of the islets is entirely to down-regulate the tendency of the islets to induce coagulation and inflammation.

We therefore state as fact that our islets, after "coating" with heparin, are not encapsulated.

Our heparin-modified islets can be obtained by simple mixing of heparin or heparin complex with the islets, as in Example 3 of our U.S. patent application. On the other hand, in Wagner and Soon-Shiong there are required operations, which are the main focus of these documents, for the creation of the capsule shells, e.g. extrusion in a two-phase coaxial flow system according to Example 20 of Soon-Shiong, or an emulsification technique with a photo polymerization as set forth in Example 19 of Soon-Shiong.

The third citation relied upon by the U.S. examiner is a publication in the name of Lenschow et al. This publication is in certain respects even more remote from our invention than are Wagner and Soon-Shiong, because the authors simply administered CTLA4Ig to mice rather than treating the islets, i.e. the CTLA4Ig was administered systemically to the

mice and not to the islets. The Lenschow et al publication therefore has nothing to do with our invention.

I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 2004-02-24 By _____
Olle Korsgren

Date: 2004-02-23 By _____
Bo Nilsson

Date: 2004-02-23 By _____
Rolf Larsson

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Applicant: Olle Korsgren

Curriculum vitae

Biografical sketch:

Name: Olle Korsgren

Date and place of birth: June 19, 1959, Falun, Sweden

Social security number: 590619 7214

Family: married, 6 children

Home address: Tallmovägen 2A, S-756 45 Uppsala, Sweden

Education:

Uppsala university, Uppsala, Sweden	Bachelor of Medicine	1982
Uppsala university, Uppsala, Sweden	M.D.	1986
Uppsala university, Uppsala, Sweden	Ph. D.	1991
Uppsala university, Uppsala, Sweden	Assist. Prof.	1994
National Board of Health and Welfare	License to practice Medicine	1994
National Board of Health and Welfare	Specialist in Clinical Immunology	2001

During my period as a medical student, 1982-1986, I attended several different university courses, corresponding to 68 weeks of full-time studies.

Research and professional experience:

Positions held:

1986-1988	Ph.D. Student	Fellowship, Uppsala University
1988-1991	Ph.D. Student	Fellowship, Swedish Medical Research Council
1991	Postdoc Research	Fellowship, Uppsala University
1992-1994	Intern	Uppsala University Hospital
1994-2000	Resident	Clinical Immunology, Uppsala University Hospital
1996-2000	Research Career Award	Swedish Medical Research Council
2001-	Senior Researeh position, (6 years, halftime) Swedish Medical Research Council	
2001-	Senior staff member Dept of Clinical Immunology, University Hospital, Uppsala	
2002-	Professor of Transplantation Immunology, Uppsala university	

Administrative experience:

1986-1991	Member of the Medical Faculty Board, Uppsala University
1989-1991	Member of the Board, Dept. of Medical Cell Biology
1997-1998	Member of the Board, Dept. of Clinical Immunology
1998-2000	Member of the Board, Swedish Transplantation Society
1999-	Member of the Editorial board Xenotransplantation
1999-	Member of the Editorial board Transplantation

Nilsson, SI Bo **MD, PhD**

Degrees

B. of Med.	1976	Uppsala university, Sweden
M.D.	1986	Uppsala university
Ph.D.	1986	Exp Clin Chemistry, Uppsala university,
Assist. Prof (Docent)	1990	Exp Clin Chemistry Uppsala university
Specialist	1991	Clinical Immunology, University Hospital, Uppsala

Society Memberships

Member of the Scandinavian Society of Immunology
Member of the Swedish Medical Society
Member of the International Complement Society

Awards and stipends

Clinical research stipend	1994-2003	Total 30 month
Novo nordisk Research stipend	2000-2003	Total 18 month (3 x 6 month)

Positions held:

1980-1986	Ph.D. Student	Fellowship, Uppsala University
1987-1988	Internship	University Hospital, Uppsala
1989-1991	Residency	University Hospital, Uppsala
1992-1993	Registrar	University Hospital, Uppsala
1993-	Chief Physician	University Hospital, Uppsala

Administrative experience:

1992-1995	Member of the Board, Dept. of Clin Laboratories
1994-	Member of the Board, Dept. of Clinical Immunology
1998-	Member of the Board of External quality assurance in laboratory medicine in Sweden (in clinical immunology)
1999-	Treasurer, Swedish Clinical Immunology Society
1999-	Member of the Board of the Swedish Expert group for Clinical Immunology
1999-2001	Head of the autoimmune immunochemistry unit, Dept Clin immunol, University Hospital, Uppsala

Relevant Publications (selected out of 116)

1. Bennet W, Sundberg B, Groth CG, Brendel MD, Brandhorst D, Brandhorst H, Bretzel RG, Elgue G, Larsson R, Nilsson B, Korsgren O. Incompatibility between human blood and isolated islets of Langerhans: a finding with implications for clinical intraportal islet transplantation? Diabetes 1999;48(10):1907-1914.
2. Özmen, L., Nilsson Ekdahl. K., Elgue, G., Larsson, L., Korsgren, O., Nilsson, B (2002). Inhibition of thrombin with Melagatran abrogates instant blood mediated islet reaction (IBMIR) in vitro: Possible application in clinical allogeneic islet transplantation. Diabetes 51 (6):
3. Moberg, L., Lukinius, A., Johansson, H., Elgue, G., Nilsson Ekdahl, K., Korsgren, O., Nilsson, B. (2002) Expression and secretion of tissue factor in the islets of Langerhans: A likely explanation for the development of the thrombotic reactions in clinical islet transplantation. Lancet Dec 21-28;360(9350):2039-45
4. Johansson U, Olsson A, Gabrielsson S, Nilsson B, Korsgren O. Cytokines and cytokine receptors cDNA array analyses of isolated human islets. Biochem Biophys Res Commun 2003;29:474-479.

Curriculum vitae

Rolf Larsson (460128-5531)

Education and academic degrees

1972 Chalmers University of Technology, Gothenburg, Sweden. M. Sci in Chemical Engineering

1980 Karolinska Institute, Dept of Experimental Surgery, Stockholm, Sweden. by the thesis " Chemical constitution and biological characteristics of a heparin surface".

1994 Uppsala university, Dept of Clinical Immunology, Uppsala, Sweden. Associate Professor in Experimental Biomaterials Research

1996 Uppsala university, Dept of Clinical Immunology, Uppsala, Sweden. Adjunct Professor in Experimental Biomaterials Research

Positions held:

1996 -	Senior Research Position	University Hospital, Uppsala
1991 -	Research Director	Corline Systems AB, Uppsala, Sweden
1984 – 1991	Research Scientist	Pharmacia AB, Uppsala, Sweden
1972 – 1984	Research engineger	Incentive Research & Development AB

Present position

1. Adj. Prof. Experimental Biomaterials Reseach, Dept of Oncology, Radiology and Clinical Immunology, Uppsala University since 1996. Assignment 40%.
2. Research Director of Corline Systems AB (permanent position).

Bibliography, last 4 years

Dissertations which have involved supervision

1. Jaan Hong. Investigation of Incompatibility Reactions Caused by Biomaterials in Contact with Whole Blood Using a New in vitro Model. Dissertation Uppsala University. 2001

2. Jonas Andersson. Complement Activation Triggered by Biomaterial Surfaces. Dissertation Uppsala University. 2003.

3. Matilda Johnell. Monocytes, Tissue Factor and Heparin-coated Surfaces. Clinical and Experimental Studies. Dissertation Uppsala University. 2003.

Original papers

1 Hong, J., Nilsson Ekdahl, K., Reynolds, H., Larsson, R., Nilsson, B. A new in vitro model to study interaction between whole blood and biomaterials. Studies of platelet and coagulation activation and the effect of aspirin (1999) Biomaterials (1999) 20: 603-11.

2 Hong, J., Larsson, A., Nilsson Ekdahl, K., Elgue, G., Larsson, R, and Nilsson, B.: Contact between a polymer and whole blood: The sequence of events leading to thrombin generation. J. Lab. Clin. Med. 2001, 138, 139-45

3 Hong J, Andersson J, Nilsson Ekdahl K, Elgue G, Axén N, Larsson R and Nilsson B: Titanium is a highly thrombogenic biomaterial: Possible implications for osteoneogenesis. (1999) Thromb. Haemostas. 82:58-64

4 Nilsson, B., Hong, J., Larsson, R., Elgue, G., Nilsson Ekdahl, K., Sahu, A, & Lambris, J. D. Compstatin inhibits complement and cellular activation in whole blood in models for extracorpereal circulation (1998) Blood 92: 1661-1667.

Review papers

1. van der Giessen, W.; van Beusekom, H.M.M.; Larsson, R.; Serruys, P.W. Heparin-coated Coronary Stents. Curr. Interventional Cardiol. Reports. 1999. 1, 234-40.

2. Larsson, R.: Heparin-binding to improve biocompatibility. In Encyclopedia for Biomaterials and Bioengineering, Marcel Dekker Inc. 2003, In press.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

ATTY.'S DOCKET: KORSGREN=1

In re Application of:	)	Art Unit: 1614
	)	
Olle KORSGREN et al	)	Examiner: Donna JAGOE
	)	
Appln. No.: 09/890,936	)	Washington, D.C.
	)	
Date Filed: November 7, 2001	)	Confirmation No. 9165
	)	
For: NOVEL USE WITHIN TRANSPLANTATION SURGERY	)	

SECOND DECLARATION UNDER 37 CFR 1.132

Olle Korsgren, Bo Nilsson and Rolf Larsson hereby each solemnly declare as follows:

We are co-applicants and co-inventors of the above-identified application. We each have both M.D. and Ph.D. degrees. We earlier submitted a declaration in this application, also under 37 CFR 1.132, which we reiterate and confirm, and which has attached thereto our CV's which are made a part of this declaration as well.

Each of us confirms familiarity with the prior art documents cited and relied upon by the U.S. patent examiner and the commentary of the U.S. examiner in conjunction with reliance on such prior art citations. We amplify our earlier declaration as follows:

Encapsulation (also referred to as microencapsulation) implies that the islets are confined

within a polymer membrane that is not in direct contact with the islets. Extensive efforts have been spent on preparing microcapsules primarily using alginates as the capsules-forming material in accordance with what is disclosed in Wagner and Soon-Shiong.

A recent article (ref. 1), Dufrane et al[1], is representative for the current state-of-the-art. As illustrated in Fig 1 in the cited article, an optimal capsule should be a sphere with a diameter of 650- 700 µm. Wagner specifies a diameter of 500 µm (p. 6, item 2, l.3-4 in translated document).

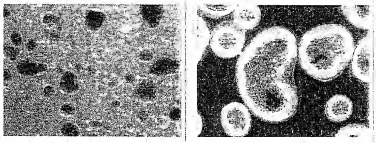
Considering that the islets have a size of 50-300 µm, we state as fact beyond any doubt that one or several islets will be enclosed in each microcapsule with considerable dead space between the islet(s) and the enclosing membrane.

Even with the most advanced technology using polyethylene glycol as the capsule-forming material, as being used by e.g. Novocell Inc. (see www.novocell.com), the capsules of the prior art leave a dead space of 25-50 µm between the cell surface and the polymer membrane (see fig. 1 below). The dead space creates delayed response times due to the fact that glucose must first diffuse through the membrane,

[1] 1. Dufrane D., Goebbels R-M., Saliez A., Guiot Y., and Gianello P.: Six-months survival of microencapsulated pig islets and alginate biocompatibility in primates. Proof-of-concept. Transplantation, 8, 1345-1353, 2006

and then the glucose has to be transported by a concentration gradient across the dead space until it reaches the cell surface.

Fig 1. (microphotographs obtained at www.novocell.com)



Purified Human Islets

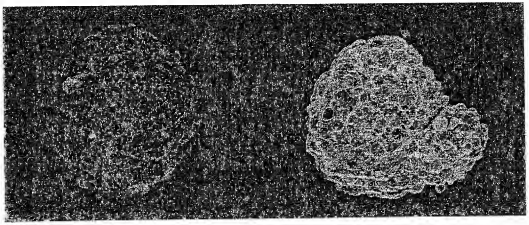
Encapsulated Islets with PEG Conformal Coatings

The insulin response by the cell will be delayed and the occurrence of insulin in the blood is further delayed by the dead space and diffusion through the membrane. The end result is that the actual insulin response in blood will be too late and may even induce hypoglycemia, if increased levels of insulin are generated too late when the glucose levels are already going down.

On the contrary, in our invention, the material used, e.g. Corline Heparin Conjugate, is adsorbed directly in close contact with individual islets with no dead space, whereby glucose in blood interacts directly with the islet cells so that a physiological response with regard to release of insulin is achieved. The following picture (Fig. 2), prepared in our laboratory, shows one islet cell coated with

heparin according to our invention and examined by confocal microscopy using fluorescently labelled antithrombin that binds to heparin. It is evident that the coating follows the contour of the cell and is in direct contact with the cell surface.

Fig. 2



Islet of Langerhans, non-modified (left) and coated with the Corline Heparin Surface (right). Antithrombin (which has strong affinity for heparin) labelled with a fluorescent dye, Alexa 488, was allowed to adsorb to the surface. Islets were then examined in a Zeiss LSM 510 Meta confocal microscope (Carl Zeiss, Jena, Germany) equipped with an Axiovert 200 microscope stand. Z-stacks of the islet surfaces were acquired using the 488nm laser line, a 20x objective and a 505-550 BP filter. Three-dimensional projections of the acquired z-stacks were analysed using Imaris software (Bitplane, Zurich, Switzerland). The non-modified islet (left) only displays weak autofluorescent, whereas the adsorbed heparin is

visualised as a smooth and coherent coating that follows the contours of the islet.

There is another distinct difference between encapsulated islets such as those of Wagner and Soon-Shiong, and heparin coated islets according to our invention that needs to be emphasised. The spherical membrane that encapsulates the islets of the prior art is an insoluble polymer with a cross-longitudinal network of bonds (thus it forms a membrane barrier between the islet surface and the surroundings), whereas the material we use, e.g. Corline Heparin Conjugate, is a soluble macromolecule that is adsorbed as discrete molecules onto the cell surface with no cross-longitudinal linkages being formed between the conjugate molecules (thus no membrane barrier is formed between the islet surface and the surroundings).

Soon-Shiong et al discusses "microcapsules prepared from crosslinkable polysaccharides, polycations and/or lipids and use therefor" (title of the patent). No part of the Soon-Shiong document demonstrates or discusses the use of heparin or heparin conjugates for coating islets without polymerization (or crosslinking, alternatively gel formation). On the contrary, examples 1-7, 14-17, 19, 21-25, 27-28 and 30-32 are specifically related to crosslinking, gels, polymerization etc. Therefore, Soon-Shiong et al do something

quite different from our invention, and this document leads away from our invention.

In their patent application, Wagner et al mention islets of Langerhans in Claim 3 and heparin used to antagonize agglomeration in claim 7. However, no disclosure of our present invention can be found in the patent application of Wagner et al. On the contrary, Wagner et al only describe the use of macro- and micro-encapsulation of islet cells, and thereby leads or directs worker in our field away from our invention.

As clearly explained in our previous Declaration, the encapsulating membrane composed of an insoluble polymer proposed by Wagner et al and Soon-Shiong et al constitutes a barrier to immunologically active cells and molecules, whereas no such barrier is created by adsorption of the heparin, e.g. Corline Heparin Conjugate. We reiterate with strong emphasis that it is absolutely clear and certain that the chemical constitution and diffusion characteristics are fundamentally different between the two methods, and that there is no chemical or technical rationale on basis of which one could accurately maintain that adsorption of the Corline Heparin Conjugate would lead to encapsulation in the sense of Wagner et al and Soon-Shiong et al.

We hereby further declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 04/02/2007 By _____
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Six-Month Survival of Microencapsulated Pig Islets and Alginate Biocompatibility in Primates: Proof of Concept

Denis Dufrane,[1] Rose-Marie Goebbels,[1] Alain Saliez,[1] Yves Guiot,[2] and Pierre Gianello[1,3]

Background. Pig islets xenotransplantation remains associated with a strong humoral and cellular xenogeneic immune responses. The aim of this study was to assess the long-term biocompatibility of alginate encapsulated pig islets after transplantation in primates

Methods. Adult pig islets encapsulated in alginate under optimal conditions (n=7) or not (n=5) were transplanted under the kidney capsule of nondiabetic Cynomolgus maccacus. Additional primates received empty capsules (n=1) and nonencapsulated pig islets (n=2) as controls. Capsule integrity, cellular overgrowth, pig islet survival, porcine C-peptide and anti-pig IgM/IgG antibodies were examined up to 6 months after implantation

Results. Nonencapsulated islets and islets encapsulated in nonoptimal capsules were rapidly destroyed. In seven primates receiving perfectly encapsulated pig islets, part of the islets survived up to 6 months after implantation without immunosuppression. Porcine C-peptide was detected after 1 month in 71% of the animals. The majority of grafts (86%) were intact and completely free of cellular overgrowth or capsule fibrosis. Explanted capsules, after 135 (n=2/2) and 180 (n=2/3) days, demonstrated residual insulin content and responses to glucose challenge (stimulation index of 2.2). Partial islet survival was obtained despite an elicited anti-pig IgG humoral response

Conclusions. Optimal alginate encapsulation significantly prolonged adult pig islet survival into primates for up to 6 months, even in the presence of antibody response.

Keywords: Pig islet transplantation, Xenotransplantation, Microencapsulation, Primate, Biocompatibility.

(*Transplantation* 2006;81: 1345–1353)

The need for permanent immunosuppression and the shortage of pancreas donors remain major hurdles to the widespread use of pancreatic islet cell transplantation to curing type 1 diabetes (*1, 2*).

In order to protect islet cells from immune reaction without the need for chronic immunosuppression, microencapsulation has been considered and demonstrated to be effective in mice (*3–6*). In most studies, islets have been encased in alginate-polylysine-alginate microcapsules (*7*) but the cytotoxicity of polyaminoacid and the mechanical instability of microcapsules limit their application (*5, 8, 9*). Several groups have recently reported that encapsulation in a simple microbead made of alginate is able to protect pig pancreatic cells against allo- or xenorejection in diabetic mice (*4, 5, 10, 11*). However, a question remains about the putative application of this method in a preclinical large animal model.

In order to overcome the shortage of human pancreases, pig islets represent an important therapeutic alternative (*12*). Although the strong humoral and cellular xenogeneic immune responses appear difficult to overcome (*13*), pig islet survival has been demonstrated for up to 53 days in primates by using anti-thymocyte globulins (*14*). Recent results reported by B. Hering (personal communication) seem, however, to show that the use of a heavy immunosuppressive regimen (5 or 6 drugs), allows pig islets to survive in primates for up to months. Such an immunosuppressive regimen seems unlikely to be acceptable in humans, but these results are, however, encouraging. This result suggests that other alternatives such as microencapsulation should be evaluated in the pig to primate model.

This study is the first to assess the factors involved in successful pig islet immunoprotection by alginate capsule when transplanted in non-diabetic primates without any immunosuppression. The impact of several parameters (stabilization, culture period) involved in in vivo and in vitro alginate capsule biocompatibility, as well as the ability of alginate capsules to protect pig islets in primates from the strong xe-

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nogeneic immune response were evaluated for up to six months.

MATERIALS AND METHODS

Pig Islet Cell Isolation and Encapsulation

Adult pig pancreases were harvested from Landrace pigs (>200 kg, n=10) at the local slaughterhouse (Centre A. de Marbaix, Louvain-la-Neuve, Mr. Collignon) and islets were isolated using a previously described technique (*15*).

Freshly isolated pig islets were encapsulated in an SLM 100 alginate matrix (Batch 110064, FMC BioPolymer, Drammen, Norway) containing a high concentration of mannuronic acid (High-M, 56%). Freeze-dried alginate (viscosity: 174 mPa.s; endotoxin <25 EU/gram), was diluted in a MOPS 1X washing buffer (Inotech Encapsulation AG, Dottikon, Switzerland) at a concentration of 1% w/v. Pig islet cells were suspended in alginate at a concentration of 10,000 islet cells/ml and encapsulation was performed by the Inotech Encapsulation AG device (serial number: LS-01.005; Dottikon, Switzerland) (Dufrane et al., manuscript submitted). Quality of capsule was microscopically evaluated (on 100 capsules samples) in order to determine the capsule diameter and the percentage of non-well shaped and broken capsules.

Microcapsule Culture Condition

In order to obtain optimal stability of microcapsule, several $CaCl_2$ concentrations were tested in culture. Empty capsules were cultured in HAM-F10 medium (NV Invitrogen, Merelbeke, Belgium) and supplemented with either 0.3, 1.8, 2.5 or 5 mM $CaCl_2$ for 2, 18 and 24 hr of incubation. One hundred capsule diameters, for each series, were examined on an inverted phase contrast microscope with a calibrated grid in the eyepiece.

Animal Recipients

Non-diabetic Cynomolgus monkeys (3–6 years old; 4–7 kg, n=15) were used as recipients and were housed according to the guidelines of the Belgian Ministry of Agriculture and Animal Care. All procedures were approved by the local Ethics Committee for Animal Care of the Université Catholique de Louvain. Three experimental groups were designed: one primate received empty capsules (Negative Control, Ctrl−); 2 primates were transplanted with non-encapsulated pig islets (Positive Control, Ctrl+) and 12 primates were transplanted with pig encapsulated islets (Experimental Group).

In this latter group, two primates received pig encapsulated islets which had not been cultivated for 18 hr prior to transplantation (without capsule stabilization), three animals received pig encapsulated islets which had been cultivated for 18 hr prior to transplantation but in medium supplemented by 10% v/v of FBS (NV Invitrogen, Merelbeke, Belgium), and finally, seven primates received pig encapsulated islets after an 18 hr period of culture in a medium free of serum.

Transplantation of Microencapsulated Islets

Kidney subcapsular space was chosen since it seems a less immunoreactive implantation site for encapsulated xenoislets than peritoneum (Dufrane et al., September 2005, submitted in Biomaterials).

After anesthesia, kidneys were exposed, by a midline laparotomy, and a 20-gauge catheter (Becton Dickinson, Aalst, Belgium) was placed between the kidney parenchyma and the capsule. Each animal received 15,000 microencapsulated islets equivalent to (IEQ)/kg of body recipient, collected in a 10 ml syringe. Positive control animals received the same number of non-encapsulated pig islets under the kidney capsule. Negative control animal received a volume of empty capsules corresponding to the volume of 15,000 IEQ/kg encapsulated pig islets (a mean of 7 ml). Each graft was transplanted under the capsule of one kidney per primate.

Animal Follow-up

Blood glucose was monitored during the first 24 hr posttransplantation to avoid hypoglycaemia. Blood samples were taken (via the femoral vein) prior to transplantation (three times to determine the Porcine C-peptide baseline) and 1 hr, days 1, 7, 30, 60, 90, 135 and 180 posttransplantation. Porcine C-peptide (Linco Research, Nuclilab BV, BB EDE, The Netherlands), anti-porcine antibodies (see below) and creatinine (Kodak Ektachem DTSC II; Ortho-Clinical Diagnostics, INC, Rochester, NY, USA) levels were measured on primate sera. Body Weight was monitored weekly.

Detection of Anti-porcine IgM and IgG

Porcine lymphocytes were used as antigenic targets for the detection of anti-porcine antibodies. Ten milliliters of heparinized blood, obtained from Belgium landrace pigs (12–15 weeks old), were diluted in 25 ml of RPMI and lymphocytes were recovered after centrifugation across a density gradient on lymphocyte separation medium (LSM; Biochrom AG, Berlin, Germany). Specific anti-porcine IgM or IgG antibodies were assessed by Flow Cytometry using a fluorescence-activated cell sorter (Facsort, Becton Dickinson, BD Erembodegem, Aalst, Belgium). The 8×10^6 porcine lymphocytes/tube were incubated 30 min with 20 μl of serum from each experimental primate (primate serum was used without dilution and decomplemented at 56°C). After incubation, at 4°C, three washes were performed with HBSS solution. FITC-labeled rat anti-human IgM (LO-BM2) and IgG (LO-HG22) antibodies (IMEX, Université Catholique de Louvain, Brussels, Belgium) were incubated with lymphocytes and washed three times after 30 min of incubation. These data were acquired and processed using CELL Quest software (BD Bioscience). The mean fluorescence intensity (MFI) and percentage of lymphocyte binding anti-porcine antibody were assessed.

Assessment of Explanted Capsules

Under anesthesia, nephrectomy was performed and the microcapsules were recovered 30, 90, 135 and 180 days after transplantation. Macroscopic quality of the graft was assessed as the following score in 3 groups: no graft fibrosis (0%); >50% of entrapped capsules in fibrosis and total graft fibrosis (100%). The proportion of capsule overgrowth, which was defined as the percentage of capsules with more than 50% of surface covered by cells, was microscopically evaluated on a mean of 400 capsules/explanted graft. The proportion of broken capsules and the proportion of capsules containing dithyzone (DTZ) staining islets were also evaluated microscopically.

The ability of encapsulated adult pig islets to produce insulin 135 and 180 days after transplantation was assessed by a static glucose challenge. Prior to the test, explanted capsules had been cultivated in 75 cm^2 flasks, for 15 hr, on RPMI 1640 glucose 5 mM supplemented with 10% FBS at 37°C. After culture, encapsulated pig islets were removed from the flasks, washed three times with RPMI 1640 glucose 5 mM and incubated for 24 hr, in RPMI 1640 supplemented with glucose 5 mM, 15 mM or 15 mM + Forskolin 1 μM (37°C). After incubation, supernatants were collected and stored at −20°C until measurement of insulin by a double antibody radioimmunoassay. Insulin content of the microencapsulated pig islets was measured in samples from each recipient. Microcapsules were added to 500 μl acid-ethanol solution, sonicated and kept at −20°C. Results of insulin secretion are given in percentage of insulin content per islet. Insulin secretion activity and insulin content were compared with the preimplantation values.

Statistics

Values are presented as means ± SD (excepted when specified otherwise). The statistical significance of differences was tested by a one-way analysis of variance (ANOVA) with a Bonferroni posthoc test. The Mann-Whitney *U* test was used to analyze the insulin secretion of explanted capsules. The statistical tests were carried out using Systat version 8.0. Differences were considered to be significant at $P<0.05$.

RESULTS

Parameters Conditioning "In Vitro and In Vivo" Biocompatibility

Size of Microcapsules and $CaCl_2$ Concentration

After encapsulation, the mean diameter of empty capsules was 633±22 μm (n=460 from three independent experiments). Incubation of capsules for 18 or 24 hr in a culture medium supplemented with 0.3 mM $CaCl_2$ provoked a sig-

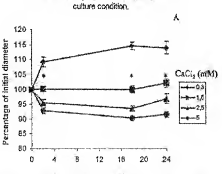

FIGURE 1. (A) Stabilization of the capsule diameter prior to transplantation. Stable capsule diameters were obtained in a medium containing 1.8 mM CaCl2 with an incubation time of 18 hr (*$P<0.005$ for capsule diameter with 1.8 mM vs. 0.3/2.5/5 mM $CaCl_2$). Significant swelling and retraction were observed in capsules incubated in medium containing low (0.3 mM) and high (2.5/5 mM) concentrations of $CaCl_2$ (300 capsules were evaluated for each series from three independent experiments), respectively. Transplantation of non-optimal (B) and optimal (C) encapsulated pig islets beneath the kidney capsule (location bar=100 μm). Note that graft fibrosis (D) and strong fibrosis (*) reaction, surrounding non-optimal capsules, were 1 month after implantation. No graft fibrosis and free-capsules were observed, 1 month after transplantation, in case of optimal capsules transplantation (E). In addition, insulin positive cells were found, at day 30 posttransplantation, inside optimal capsules (G; immunostaining for anti-insulin; original magnification: ×2.5) in contrast to capsules from non-optimal conditions (F, no staining for insulin; original magnification: ×2.5).

TABLE 1. Scoring of encapsulated pig islets after explantation

Animal number	Time of graft explantation	Macroscopic evaluation of graft (% of fibrosis)	Microscopic evaluation of graft (% of total capsule)			Porcine C-peptide (ng/ml) at day 30
			Degree of capsule overgrowth	Degree of broken capsule	Capsule containing DTZ* cells	
Non-optimal encapsulation						
FBS						
001	30	100	ND	ND	ND	0
002	30	100	ND	ND	ND	0
003	30	100	ND	ND	ND	0
Without preimplant 18-hr culture						
004	30	50	94	86	0	0.04
005	30	100	ND	ND	ND	0.03
Optimal encapsulation						
006	30	0	3	2	54	0.119
007	90	0	2	6	32	0.106
008	135	0	1	0	17	0.288
009	135	0	6	4	12	0.157
010	180	0	5	0	6	0.199
011	180	50	32	26	0	0.049
012	180	0	8	12	3	0.053

Capsules were removed, from the primate's kidney 30, 90, 135 and 180 days after transplantation. Two independent investigators evaluated the grafts. The degree of cellular overgrowth, broken capsules and DTZ contained islets were microscopically evaluated on 400 capsules for each graft. ND, nondetermined since all the grafts were entrapped in fibrosis.

nificant swelling of capsules (+12.5% of initial diameter prior to culture (745±42 μm), $P<0.005$) (Fig. 1A). No significant modification of the diameter was observed for capsules incubated in culture medium supplemented with 1.8 mM $CaCl_2$ for 2 h (703±32 μm), 18 h (698±36 μm) or 24 hrs (707±69 μm). In contrast, incubation in medium with higher $CaCl_2$ concentrations (2.5 and 5.0 mM) induced a significant reduction in capsule diameter (−6.6%, $P<0.005$) as compared to the diameter obtained just after encapsulation.

Culture of Microencapsulated Islets Prior to Implantation

In order to stabilize the microcapsules, and obtain long term biocompatibility of encapsulated pig islets, preimplantation culture for 18 or 24 hr is necessary. Two primates that were immediately implanted after encapsulation demonstrated a strong cellular/fibrosis reaction against the graft after 1 month (Table 1, animals 004-005).

Three animals which received pig encapsulated islets cultivated prior to transplantation in CMRL 1066 supplemented with bovine serum, encapsulated islets were rapidly destroyed (<30 days) and massive cellular overgrowth and fibrosis were observed (animals 001-3) (Table 1; Fig. 1, D and F).

In these five primates, no porcine C-peptide was detected at day 30 posttransplantation (Table 1, Fig. 2). In contrast, primates transplanted with capsules cultured in optimal conditions demonstrated the biocompatibility of alginate encapsulated pig islets (see below, Table 1; Fig. 1, E and G).

Shape of Microcapsules

In order to obtain a long term biocompatibility of capsule, a regular, spherical shape of capsule is necessary (Table 1, Fig. 1C). One primate (animal 011) that received pig islets in capsules 38% of which were not well formed (Fig. 1B) displayed significant fibrosis (>50%) after 6 months. In this case, cellular overgrowth (32%) and capsular breakage (26%) were observed (Table 1). In the other primates that received a mean of 91±5% of well-formed capsules (Fig. 1C), fibrosis or cellular overgrowth was not evidenced.

A clear relationship between the capsule shape and the purity of islets preparations was evidenced. The exocrine contamination disturbs the laminar jet of the encapsulation device thereby increasing the proportion of non-well shaped capsules. In this study, the purity was constantly over or equal to 90% (94±2.6%: n=10) to be able to use our device.

Ideal Preparation of Alginate Microencapsulated Pig Islets

These preliminary data allowed us to determine the following best condition for in vivo experiments: the best regime was to culture pig encapsulated islets in 17 ml CMRL 1066 at 1.8 mM $CaCl_2$ for 18 hr, in 75 cm^2 non-tissue culture treated flask at a concentration of 10,000 capsules/ flask in a serum-free medium. In 6 out of 7 remaining primates, no graft fibrosis or cellular overgrowth was observed after 3, 4 or 6 months whether the microencapsulated pig islets were cultivated in the appropriate medium and when over 92% of capsules were well formed (Table 1).

Transplantation of Non-encapsulated Pig Islets and Empty Capsules

After transplantation of non-encapsulated pig islets under the kidney capsule of 2 primates, a peak of porcine C-peptide was evidenced 1 hr after transplantation (range 2,319–6,122 ng/ml). Porcine C-peptide was, however, below the detection threshold (<0.1 ng/ml) 2 days after transplantation (Fig. 2). After graft removal and tissue fixation, a strong

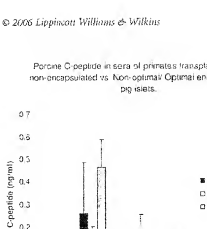

FIGURE 2. Porcine C-peptide level evolution in primates transplanted with non-encapsulated pig islets (n=2; Black) or non-optimal (n=5, White) / optimal (n=7; Grey) encapsulated pig islets. After 30 days of optimal capsules transplantation, porcine C-peptide was detected in 71% of transplanted animals (5/7 primates). No porcine C-peptide was detected 2 days after the implantation of free and non-optimal encapsulated pig islets (<0.1 ng/ml, black line). Mean ± SEM.

cellular reaction was observed 2 and 7 days after transplantation (Fig. 3). The cellular infiltration was essentially composed of CD3 (lymphocyte) and CD68 (monocyte-macrophage) positive cells (Fig. 3, B and C). Complement C3 deposition was evidenced on the graft, as soon as 2 days after transplantation (Fig. 3D). Empty capsules did not induced inflammatory and immunological reactions (data not shown).

Encapsulation Prolongs Pig Islet Survival

After stabilization, encapsulation of pig islets by high-M alginate improved graft survival after transplantation into non-diabetic primates (n=7). A mean level of 0.14±0.08 ng/ml of porcine C-peptide was detected until day 30 post-transplantation, in the sera of 7 primates. However, 2 primates had only a detectable level of porcine C-peptide (>0.1 ng/ml) until 7 days after transplantation (Fig. 2, Table 1). Level of C-peptide was significantly higher than the level obtained in naive animals (0.03±0.02 ng/ml). Dithizone and insulin positive cells were found in one graft after removal at day 30 posttransplantation (n=1). No fibrosis and no cellular overgrowth were observed in this graft (Table 1). Porcine C-peptide was detected in 2 recipients after 60 days of transplantation (0.206 and 0.193 ng/ml). Although no porcine C-peptide was detected in primate sera over 90 days posttransplantation, no graft fibrosis, no capsule overgrowth and insulin positive cells were observed (Table 1). After 135 (n=2) and 180 (n=3) days of transplantation, no graft fibrosis and fewer than 10% of capsules presenting cellular overgrowth were observed in 5 out of 6 primates (Table 1, Fig. 4, A and B). Dithizone positive cells were found inside grafts after 135 and 180 days of transplantation (Table 1, Fig. 4, C and D).

No significant modification of body weight and creatinine level was observed in the 7 primates receiving well encapsulated pig islets 4 to 6 months after implantation.

Microencapsulated Pig Islets Are Viable and Functional 4 and 6 Months after Implantation

Prior to transplantation, a mean viability of 84±6% (by Trypan Blue exclusion assay) was obtained for encapsulated pig islets (n=10). Transmission electron microscopy demonstrated the integrity of pig islets after encapsulation (data not shown). Prior to implantation, encapsulated pig islets (islets from 3 pig pancreases used for animals 008-12) demonstrated the capacity to produce insulin after glucose stimulation: an increased insulin release after exposure to glucose 15 mM supplemented with cAMP-raising agent (Forskolin 1 μM) was observed: 70.7±15.1% vs. 31.6±9.7% of insulin content for glucose 15 mM+Fsk 1 μM vs. glucose 5 mM, respectively. The mean stimulation index (SI), calculated as the fold-increase over baseline (% of insulin content at glucose 5 mM), was calculated at 2.5±1.1.

Capsules were removed 135 (n=2) and 180 (n=3) days after transplantation and were incubated in the presence of different concentrations of glucose to assess the function of pig islets from explanted capsules. An increase in insulin release, after exposure to glucose 15 mM supplemented with Forskolin, was observed for pig encapsulated islets removed at day 135 (animals 008-009): 6.6±2.3% vs. 2.9±0.9% of insulin content for glucose 15 mM+Fsk 1 μM vs. glucose 5 mM ($P=0.028$, n=2; Fig. 4E). The mean SI was calculated at 2.2 (range 2.0–2.7). After 180 days of transplantation, one graft did not demonstrate insulin content and the capacity to secrete insulin. This correlated with graft fibrosis and capsule overgrowth (Table 1, animal 011). Among the 2 other cases (animals 010-012), one pig islet graft demonstrated the capacity to increase insulin secretion after exposure to glucose 15 mM+Fsk 1 μM (8.4±3.7% vs. 4.7±1.9% of insulin content for glucose 15 mM+Fsk 1 μM vs. glucose 5 mM) corresponding to a SI at 1.8 (Fig. 4F). Follow up at the same time, observed insulin secretion, but no response to increasing glucose concentrations or the cAMP-raising agent were demonstrated in the second animal.

However, a significant decrease in insulin content was observed in capsules explanted from primates after 135 (2.2±1.9 ng/islet) and 180 (1.1±1.0 ng/islet) days of transplantation ($P<0.005$) as compared to those extracted from capsules prior to transplantation (32.2±24.3 ng/islet) for capsules containing a mean of 2–3 pig islet cells, $P<0.005$).

Transplantation of Encapsulated Pig Islets Elicits a Humoral Immune Response

Sera from 12 animals transplanted with encapsulated pig islets were analyzed, by Flow Cytometry, and compared to animals receiving non-encapsulated pig islets (n=2) and empty capsules (n=1). In all primates, the presence of anti-pig antibodies (IgM and IgG) was detected prior to transplantation thereby confirming the presence of preformed anti-pig antibodies (Fig. 5). No increase in IgM or IgG anti-pig antibodies was found in the sera of primates transplanted with empty capsules. In contrast, when primates were given non-

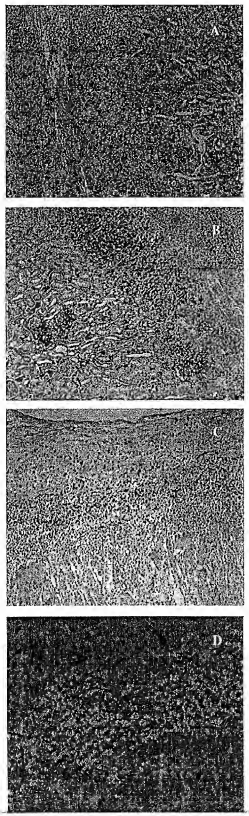

FIGURE 3. Cellular infiltration and graft destruction were observed 7 days after non-encapsulated pig islet transplantation. Very few insulin-positive cells were found ((A) insulin staining; original magnification: ×8). Immunohistochemistry revealed massive CD3 (B) and CD68 infiltration (C) (original magnification: ×8). Complement C3 deposition was observed on the graft (original magnification: ×10, D).

encapsulated pig islets ($n=2$) the level of anti-pig IgM and IgG antibodies was strongly increased. When badly encapsulated islets ($n=2$), islets cultivated in bovine serum ($n=3$) or well encapsulated pig islets ($n=7$) were implanted a significant increase in the level of anti-pig IgM and IgG antibodies was observed as shown in Figure 5.

DISCUSSION

The first aim of this study was to demonstrate the biocompatibility of encapsulated pig islets for long-term (6 months) in primates and to evidence the parameters which significantly influence the compatibility of these capsules. The second major goal was to proof the concept by demonstrating that pig encapsulated islets are capable to respond to a hyperglycemic stimulus 6 month after implantation into the most stringent xenogeneic model and without immunosuppression.

Without encapsulation, pig islets are destroyed within 2 days of transplantation as evidenced by loss of C-peptide production and humoral and cellular rejection at immunohistology (*16*).

In order to prolong porcine islets viability without immunosuppression, pig islets were encapsulated following several parameters which allowed long-term viability and immunoprotection (*17, 18*). Pig islets were encapsulated in a highly purified high-M alginate cross-linked with Ca^{++} ions and the use of a very low endotoxin-containing alginate without additional permselective PLL coating seemed crucial (*9, 19*). Similarly, Duviver-Kali et al. and Omer et al. reported improved capsule integrity and pig islet viability up 3 months after transplantation in B6AF1 mice, by using similar alginate (*10, 20*).

Although recent experiments demonstrated improved stability after alginate cross-linkage with Ba^{++}, we tested alginate capsule cross-linkage with Ca^{++} followed by a preimplantation culture at a $[Ca^{++}]$ concentration of 1.8 mM (*4, 5*). This $[Ca^{++}]$ concentration was chosen after testing several lower and higher concentrations of $CaCl_2$ during preimplantation culture up to 24 hr. Although Ham F10/F12 medium corresponds to the optimal culture media for non-encapsulated adult pig islets culture, low $[CaCl_2]$ (0.3 mM) media induce capsule swelling and provoke disruption of capsule membranes which could expose pig tissue to the host immune system and thereafter to graft fibrosis and islet necrosis (*17, 21*). Following these in vitro data, the optimal concentration of 1.8 mM was selected as well as a culture period of 18 hr prior to implantation in order to stabilize the alginate capsules. Several commercial media contain a concentration of 1.8 mM $CaCl_2$ and CMRL 1066 was chosen to culture encapsulated pig islets since its efficacy for pig islet cultures is known (*22*). In three primates, encapsulated pig islets were

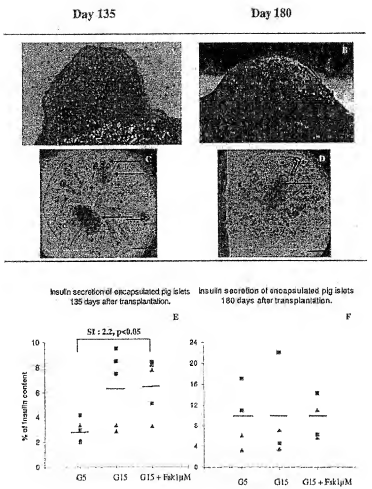

FIGURE 4. No fibrosis was observed macroscopically for grafts removed after 135 and 180 days of transplantation in primates (A, B, *graft). Dithizone positive cells (arrow) were also detected for pig encapsulated islets explanted after 135 (C) and 180 (D) days (location bar: 100 µm). Encapsulated pig islets explanted from grafts 135 days after transplantation were able to produce a significant amount of insulin after glucose/Forskolin stimulation (E), ($P<0.05$ for G15+Fsk vs. G5, n=2). After 180 days, insulin secretion was observed but without response to stimulation (F, n=2). Black square and black triangle represents replicates for each primate.

cultured in CMRL medium complemented with bovine serum, and severe graft destruction ensued one month after implantation. Although capsules were intensively washed prior to transplantation, bovine proteins could stick to the capsule surface and lead to the host immune system activation. Therefore, serum-free CMRL was then used to culture encapsulated pig islets in the following seven in vivo implantations and graft destruction was not evidenced.

The percentage of well-formed capsules also appears crucial for pig encapsulated islet biocompatibility. In fact, one primate which received stabilized encapsulated pig islets cultivated in free-serum medium, demonstrated 50% graft fibrosis 6 months after implantation. Retrospectively, this graft was composed by a high percentage (38%) of pig islets protruding outside the capsules (23).

Overall, these data suggest that encapsulated pig islets must be embedded in very pure alginate, cultivated for 18 or 24 hr in serum-free medium containing a concentration of 1.8 mM of $CaCl_2$. In addition, the ratio of well-formed capsules must be over 90% to obtain a long term in vivo biocompatibility in the pig to primate model.

Although the survival of encapsulated pig islets in diabetic monkeys was reported nine years ago but never confirmed by others teams (7), there is one recent and casuistic manuscript describing biocompatibility of alginate/polyornithine/alginate microcapsules after 8 weeks of implantation into non-diabetic primate (24). The present experimental work in vivo clearly demonstrates that implantation of optimized capsules might improved pig islet survival into primates without immunosuppression for up to 6 months. In this study, the monkeys were not diabetic since the first goal was to assess the biocompatibility of pig encapsulated islets and to prove the concept. The more important result obtained in this study was certainly the demonstration that 135 or 180 days after the implantation of encapsulated pig islets, some of the islets survived and were able to respond in vitro to a glucose challenge showing a stimulation index up to 2.2. This result demonstrates that pig islets survived up to 6 months in the most stringent xenogeneic pig to primate model without any immunosuppression. Evidently, among the explanted capsules there was a strong reduction of insulin content as compared to pre-transplantation level (from 30 ng/islet up to 2 ng/islet), but the loss of surviving beta cells probably derived from multiple origins: the fact that (i) capsules

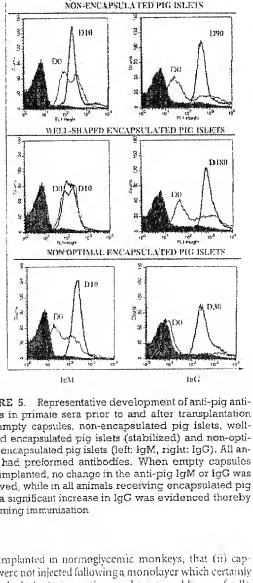

FIGURE 5. Representative development of anti-pig antibodies in primate sera prior to and after transplantation with empty capsules, non-encapsulated pig islets, well-shaped encapsulated pig islets (stabilized) and non-optimally encapsulated pig islets (left: IgM, right: IgG). All animals had preformed antibodies. When empty capsules were implanted, no change in the anti-pig IgM or IgG was observed, while in all animals receiving encapsulated pig islets a significant increase in IgG was evidenced thereby confirming immunisation.

were implanted in normoglycemic monkeys, that (ii) capsules were not injected following a monolayer which certainly induced a lack of oxygenation on the external layers of cells, that (iii) chronic rejection ensues due to continuous anti-pig IgG production or eventually, that (iv) cytokine release by encapsulated islets occurred (*18*). Regarding this latter parameter, however, it must be pointed out that cytokine release was clearly identified as a mechanism of islets loss when encapsulated islets were injected into the peritoneum which seems a more inflammatory site than kidney space (Dufrane et al., manuscript submitted).

The site implantation under the kidney capsule was, used in this work, since local oxygen pressure is high and the recovery of capsules is rendered easy for analysis, but for clinical applications other sites should be investigated i.e. as subcutaneous space. The usual intra-portal way, used in clinical applications, would be impossible due to the capsules size which will induce hepatic thrombosis.

Some of the pig islets survived long-term despite a strong humoral anti-pig immune response. In fact, all the primates used in this study had preformed anti-pig antibodies of both IgM and IgG types. Despite the encapsulation, all primates developed an elicited anti-pig immune response as evidenced by the significant shift of both anti-pig IgM and mainly IgG antibodies by flow cytometry. When empty capsules were implanted under the kidney capsules, no humoral response was elicited up to day 145 after grafting thereby demonstrating that pig islets were responsible for the antibody response. Despite this antibody production, no rejection or fibrosis was evidenced thereby demonstrating the immune protection of the pig islets by the capsules (*10*). The immunization against pig proteins could be the consequence of a small percentage of pig islets not being encapsulated or simply prove that pig proteins might get out of the capsules (*25*), such as porcine C peptide (*26*).

In conclusion, this study demonstrated that several parameters must be in place to improve the biocompatibility and survival of encapsulated pig islets up to 6 months in the most stringent xenogeneic pig to primate model. This partial but long-term survival is obtained despite an ongoing anti-pig IgG response thereby showing that encapsulation protects the islets in the long term. Similar experiments are being performed in STZ-induced diabetic monkeys to demonstrate the usefulness of this approach in type1 diabetes and the possible correction of hyperglycemia in vivo. In addition, the cellular graft is now being designed as a mono-layer graft to improve the oxygenation of beta cells.

ACKNOWLEDGMENTS

We are grateful to E. Legrand and G. Beurin for technical assistance.

REFERENCES

1. Weir GC, Bonner-Weir S. Scientific and political impediments to successful islet transplantation. *Diabetes* 1997; 46: 1247.
2. Shapiro AM, Lakey JR, Ryan EA, et al. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *N Engl J Med* 2000; 343: 230.
3. Orive G, Hernandez RM, Gascon AR, et al. Cell encapsulation: promise and progress. *Nat Med* 2003; 9: 104.
4. Omer A, Duvivier-Kali V, Fernandes J, et al. Long-term normoglycemia in rats receiving transplants with encapsulated islets. *Transplantation* 2005; 79: 52.
5. Duvivier-Kali VF, Omer A, Parent RJ, et al. Complete protection of islets against allorejection and autoimmunity by a simple barium-alginate membrane. *Diabetes* 2001; 50: 1698.
6. Schneider S, Feilen PJ, Brunnenmeier I, et al. Long-term graft function of adult rat and human islets encapsulated in novel alginate-based microcapsules after transplantation in immunocompetent phoenetic mice. *Diabetes* 2005; 54: 687.
7. Sun Y, Ma X, Zhou D, et al. Normalization of diabetes in spontaneously diabetic cynomolgus monkeys by xenografts of microencapsulated porcine islets without immunosuppression. *J Clin Invest* 1996; 98: 1417.

8. Strand BL, Ryan TL, In't VP, et al. Poly-L-Lysine induces fibrosis on alginate microcapsules via the induction of cytokines. *Cell Transplant* 2001; 10: 263.
9. King A, Sandler S, Andersson A. The effect of host factors and capsule composition on the cellular overgrowth on implanted alginate capsules. *J Biomed Mater Res* 2001; 57: 374.
10. Duvivier-Kali VF, Omer A, Lopez-Avalos MD, et al. Survival of microencapsulated adult pig islets in mice in spite of an antibody response. *Am J Transplant* 2004; 4: 1991.
11. Korbutt GS, Mallett AG, Ao Z, et al. Improved survival of microencapsulated islets during in vitro culture and enhanced metabolic function following transplantation. *Diabetologia* 2004; 47: 1816.
12. Cooper DK, Gollackner B, Sachs DH. Will the pig solve the transplantation backlog? *Annu Rev Med* 2002; 53: 133.
13. Buhler L, Deng S, O'Neil J, et al. Adult porcine islet transplantation in baboons treated with conventional immunosuppression or a non-myeloablative regimen and CD154 blockade. *Xenotransplantation* 2002; 9: 3.
14. Rijkelijkhuizen JK, Haanstra KG, Wubben J, et al. T-cell-specific immunosuppression results in more than 53 days survival of porcine islets of langerhans in the monkey. *Transplantation* 2003; 76: 1359.
15. Dufrane D, Goebbels RM, Pelliat I, et al. Impact of porcine islet size on cellular structure and engraftment after transplantation: adult versus young pigs. *Pancreas* 2005; 30: 138.
16. Kirchhof N, Shibata S, Wijkstrom M, et al. Reversal of diabetes in non-immunosuppressed rhesus macaques by intraportal porcine islet xenografts precedes acute cellular rejection. *Xenotransplantation* 2004; 11: 396.
17. Zimmermann H, Zimmermann D, Reuss R, et al. Towards a medically approved technology for alginate-based microcapsules allowing long-term immunoisolated transplantation. *J Mater Sci Mater Med* 2005; 16: 491.
18. De Vos P, Hamel AF, Tatarkiewicz K. Considerations for successful transplantation of encapsulated pancreatic islets. *Diabetologia* 2002; 45: 159.
19. Klock G, Pfeffermann A, Ryser C, et al. Biocompatibility of mannuronic acid-rich alginates. *Biomaterials* 1992; 13: 707.
20. Omer A, Duvivier-Kali VF, Trivedi N, et al. Survival and maturation of microencapsulated porcine neonatal pancreatic cell clusters transplanted into immunocompetent diabetic mice. *Diabetes* 2003; 52: 69.
21. Holmes MA, Clayton HA, Chadwick DR, et al. Functional studies of rat, porcine, and human pancreatic islets cultured in ten commercially available media. *Transplantation* 1995; 60: 854.
22. Brandhorst D, Brandhorst H, Hering BJ, Bretzel RG. Long-term survival, morphology and in vitro function of isolated pig islets under different culture conditions. *Transplantation* 1999; 67: 1533.
23. Basta G, Sarchielli P, Luca G, et al. Optimized parameters for microencapsulation of pancreatic islet cells: an in vitro study charting on islet graft immunoprotection in type 1 diabetes mellitus. *Transpl Immunol* 2004; 13: 289.
24. Elliott RB, Escobar L, Calafiore R, et al. Transplantation of Micro- and Macroencapsulated piglet islets into mice and monkeys. *Transplant Proc* 2005; 37: 466.
25. De Vos P, De Haan B, Pater J, Van Schilfgaarde R. Association between capsule diameter, adequacy of encapsulation, and survival of microencapsulated rat islet allografts. *Transplantation* 1996; 62: 893.
26. Lanza RP, Beyer AM, Chick WL. Xenogeneic humoral responses to islets transplanted in biohybrid diffusion chambers. *Transplantation* 1994; 57: 1371.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	)	Art Unit: 1614
	)	
Olle KORSGREN et al	)	Examiner: Donna Jagoe
	)	
Appln. No.: 09/890,936	)	Washington, D.C.
	)	
Date Filed: November 7, 2001	)	Confirmation No. 9165
	)	
For: NOVEL USE WITHIN TRANSPLANTATION SURGERY	)	ATTY.'S DOCKET: KORSGREN=1

THIRD DECLARATION OF ROLF LARSSON

(1) I, Rolf Larsson, hereby solemnly declare as follows:

(2) I am the same Rolf Larsson who is a co-inventor of the invention of the above-identified application, and a co-applicant of this application; and the same Rolf Larsson who executed first and second declarations respectively filed in the present application on March 2, 2004, and April 4, 2007.

(3) I confirm the accuracy of what is stated in those earlier declarations, except for one inadvertent error: while Olle Korsgren and Bo Nilsson have both M.D. and Ph.D. degrees, I do not have an M.D. degree. This was incorrectly stated in the second declaration but was correctly stated in the first declaration.

(4) I attended, along with our U.S. attorney, an interview in the United States Patent and Trademark Office on June 28, 2007. Since that time, the examiner has issued another rejection, mailed August 9, 2007, and I have studied that Office Action. In that Office Action, the examiner states that the concept of coating individual islet cells separately with a heparin coating is a concept that was not present in the specification as originally filed. This conclusion by the examiner is incorrect.

(5) The specification uses the nomenclature "isolated islets", which refers to islets being isolated from the pancreas according to well-established procedure. Such procedure produces individual islets as is readily observed by an ordinary microscope and well appreciated by workers in the present field. An individual islet is illustrated by a photomicrograph in our second declaration at page 4, Fig. 2, which was (as stated in the second declaration) prepared in our laboratory. As there stated (bottom of page 3 and top of page 4 of our second declaration), Fig. 2 "shows one islet cell coated with heparin according to our invention and examined by confocal microscopy using fluorescently labelled antithrombin that binds to heparin. It is evident that the coating follows the contour of the cell and is in direct contact with the cell surface".

(6) Based on my experience and my knowledge, I know as fact that the isolated islets are individually coated with heparin according to our invention. Furthermore, I state with utmost confidence that anyone skilled in the art will readily appreciate that "isolated islets" and "individually isolated islets" are synonymous.

(7) The new Office Action repeats rejections based on Wagner et al and on Soon-Shiong et al. I understand that these rejections are based on the examiner's position that what is recited in the claims is not new. These rejections are incorrect because our process as set forth in claims 4, 8 and 11 is new and is quite different from both Wagner and Soon-Shiong.

(8) The examiner refers to claim 8 of Wagner, but claim 8 does not disclose the use of heparin or anything similar to heparin. Claim 8 of Wagner refers to a product characterized by an immobilization system of a porous or hollow material.

(9) Claim 7 of Wagner does mention heparin (among other possibilities) "used to antagonize agglomeration." Other than claim 7, Wagner does not mention heparin. Wagner does not describe how heparin might be used in the Wagner system. The examiner says on page 8 of the Office Action that in Wagner "the islet cells are combined with heparin and

encapsulated with a polymer such as alginate", but there is no such disclosure which I have been able to find in Wagner.

(10) And on page 7, Wagner states: "Most of the microcapsules of a diameter of half a millimeter have a volume several times larger than that of the islets of Langerhans, which are 50-300 µm in size."

(11) The examiner says that "if the cells are microencapsulated, they are first mixed with anticoagulant material, thus anticipated the claims of the instant application". Not only can I find no such disclosure in Wagner, but what the examiner states is basically impossible as pointed out during the aforementioned interview. If the cells were first mixed with an anticoagulant and then encapsulated, the anticoagulant could not function because the anticoagulant would be sealed within the microcapsule.

(12) Soon-Shiong discloses, as indicated by the title of this patent, "microcapsules prepared from cross-linkable polysaccharides, polycations and/or lipids and use therefor". There is no doubt that cross-linking is intended and is provided in the formation of the microcapsule shell in Soon-Shiong. To the contrary, heparin, as well as Corline Heparin Conjugate, the preferred heparin material used in the present invention, is a water soluble substance that is adsorbed onto the islet surface as shown in Fig. 2 at page 4

of the aforementioned second declaration, and there is no cross-linking and no ability of the heparin or Corline Heparin Conjugate to cross link.

(13) While Soon-Shiong mentions heparin as a possible agent to prevent agglomeration of the microcapsules, it does not disclose the possibility that heparin may be applied directly to the surface of the islets.

(14) Newly relied upon Nomura et al discloses only the use of heparin administered systemically. Systemic administration of heparin is likely to generate bleeding complications, and has nothing to do with our invention which relates to the use of surface-bound heparin which acts locally at the surface of the islets thus eliminating bleeding complications.

(15) New applied Couser et al addresses another aspect of islet transplantation, namely systemic administration of a drug (sCR1) which is contrary to the present invention relating to the use of surface-bound heparin which acts locally at the surface of the islets.

I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are

punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 2007-11-29 By _____
Rolf LARSSON

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	)	Art Unit: 1614
	)	
Olie KORSGREN et al	)	Examiner: Donna A. Jagoe
	)	
I.A. Filing Date: 02/04/2000	)	Washington, D.C.
371(c) Date: November 7, 2001	)	
	)	Confirmation No. 9165
U.S. Appln. No.: 09/890,936	)	
	)	
For: NOVEL USE WITHIN TRANSPLANTATION SURGERY	)	ATTY.'S DOCKET: KORSGREN=1

DECLARATION OF JAMES SHAPIRO, M.D., PH.D., FRCSC

I, James Shapiro, hereby solemnly declare as follows:

I am Director, Clinical Islet Transplant Program, University of Alberta, Canada. Attached is an abbreviated form of my *Curriculum Vitae.*, which is made a part of this declaration. I have a Ph.D. degree and MD degree and have published more than 200 scientific papers including the paper from 2000 known as the Edmonton Protocol. Between 2005 and 2007, I was the President of the International Pancreas and Islet Transplantation Association.

I know Rolf Larsson, one of the inventors of the above-identified application, but I have no professional relationship with Professor Larsson or with the assignee of the above-identified U.S. patent application, Corline Systems AB. I have been asked to submit a statement with regard to the meaning of certain terms in the above-identified U.S.

patent application, as an independent expert in the field of transplantation of islets of Langerhans.

I have carefully reviewed the above-identified U.S.S patent application 09/890,936, as well as the first and second Declarations filed in the above-identified application. I have also reviewed the latest Office Action explaining the views of the Examiner.

My understanding of the Examiner's commentary is that the examiner believes that there is a difference between the terminology "individually isolated islets" and "isolated islets". But the examiner's understanding is incorrect because the two phrases, "isolated islets" and "individually isolated islets", are entirely equivalent, and refer to islets extracted from whole pancreas through mechanical and enzymatic techniques, followed by density purification to enrich the proportion of islets over the remnant contaminating exocrine cellular components.

I understand from the patent application specification of 09/890,936 that the "isolated islets" are "individually isolated islets", without any question.

It is also my professional opinion that workers in the field of islets research and related fields, including scientists involved in this area, would also understand that

the term "isolated islets" means that such "isolated islets" are "individually isolated islets."

The terms "isolated islets" and "individually isolated islets" are quite distinct from another term, islet encapsulation, the latter term of which refers specifically to immunological isolation of islets from attacking immune damaging cells.

I understand from reading the specification of the above-identified application 09/890,936 that the individually isolated islets are treated with a clotting inhibiting agent, e.g. heparin or soluble Coviline heparin conjugate, which is adsorbed onto the surface of the individual isolated islets. This adsorption of clotting inhibiting agent onto the individual isolated islets is quite different from islet encapsulation, the latter of which refers specifically to immunological isolation of islets from attacking immune damaging cells.

There is no confusion between these terms throughout the islet research field, and all scientist involved in this area would certainly comprehend the distinction between immune isolation (encapsulation) as opposed to isolated or individually isolated islets, namely islets which have been extracted from their exocrine matrix.

I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

By _____
James Shapiro, M.D., Ph.D., FRCSC

Date: 8th December 2007

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Who is in the CIT Consortium?

James Shapiro, M.D., Ph.D., FRCSC

Born in Leeds, England, Dr James Shapiro obtained his Medical Degree at the University of Newcastle-upon-Tyne and trained in Surgery at the University of Bristol. In 1993, He came to the University of Alberta in Canada to train in liver transplantation and hepatobiliary surgery, continuing research studies in experimental islet transplantation begun as a medical student. He earned a Ph.D. studying new drug combinations for possible testing in islet transplantation. He then further trained in liver surgery in Vancouver, in living donor liver transplant surgery in Japan, and in whole pancreas transplant surgery at the University of Maryland. In 1998, he returned to the University of Alberta as a multi-organ transplant surgeon.

Dr. Shapiro was asked to lead the Clinical Islet Transplant Program team in Edmonton; Together with Drs. Lakey, Ryan, Rajotte, Kneteman and Korbutt, he developed and tested a new protocol that used a steroid-free anti-rejection regimen coupled with sufficient numbers of transplanted islets. This research has since become known as the "Edmonton Protocol." In 1999, Dr. Shapiro initiated the pancreas transplant program at the University of Alberta, and in the same year performed the first emergency living-related donor liver transplant in Canada.

Dr. Shapiro is Principal Investigator of the international multi-center trial of islet transplantation testing the Edmonton Protocol at 9 international sites, sponsored by the Immune Tolerance Network. He is also Principal Investigator and Director of the Juvenile Diabetes Research Foundation (JDRF) Clinical Center for Islet Transplantation created in 2001 at the University of Alberta. In 2002, Dr. Shapiro was awarded the Canadian Institutes of Health Research/Wyeth Clinical Research Chair in Transplantation at the University of Alberta.

In 2005,Dr. Shapiro received a Meritorious Service Medal from the Governor General of Canada for his work towards the development of a new treatment for Diabetes. He was also named one of the "Physicians of the Century", by the College of Physicians and Surgeons of Alberta and the Alberta Medical Association. In 2006, he was named one of Nature Biotechnology's most remarkable and influential personalities from the past 10 years, in Biopharmaceuticals.

Dr. Shapiro maintains an active immunology/transplant research laboratory focused on aspects of tolerance induction relating to islet transplantation with emphasis on costimulatory blockade and chimerism, with translational potential to clinical islet recipients. In early 2004, Dr. Shapiro was awarded an Alberta Heritage Foundation for Medical Research Scholarship to support his on-going tolerance research.

Clinical Islet Transplant Program, University of Alberta

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